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## Past-President's contribution / Contribution du président sortant

## Genetic engineering of plants to enhance resistance to fungal pathogens—a review of progress and future prospects

Zamir K. Punja

**Abstract:** Recent applications of techniques in plant molecular biology and biotechnology to the study of host-pathogen interactions have resulted in the identification and cloning of numerous genes involved in the defense responses of plants following pathogen infection. These include: genes that express proteins, peptides, or antimicrobial compounds that are directly toxic to pathogens or that reduce their growth in situ; gene products that directly inhibit pathogen virulence products or enhance plant structural defense genes, that directly or indirectly activate general plant defense responses; and resistance genes involved in the hypersensitive response and in the interactions with avirulence factors. The introduction and expression of these genes, as well as of antimicrobial genes from nonplant sources, in a range of transgenic plant species have shown that the development of fungal pathogens can be significantly reduced. The extent of disease reduction varies with the strategy employed as well as with the characteristics of the fungal pathogen, and disease control has never been complete. Manipulation of salicylic acid, ethylene, and cytokinin levels in transgenic plants have provided some interesting results with regard to enhanced disease tolerance or susceptibility. The complex interactions among the expressed gene product, plant species, and fungal pathogen indicate that the response of transgenic plants cannot be readily predicted. Combinations of defense gene products have shown considerably more promise in reducing disease than single-transgene introductions. The use of tissue-specific or pathogen-inducible promoters, and the engineered expression of resistance genes, synthetic antimicrobial peptides, and elicitor molecules that induce defense responses have the potential to provide commercially useful broad-spectrum disease resistance in the not-too-distant future. The issues and challenges that will need to be addressed prior to the widespread utilization of these transgenic plants are highlighted.

**Key words:** antifungal proteins, antimicrobial peptides, biotechnology, elicitors, hypersensitive response, pathogenesis-related proteins, phytoalexins, resistance genes, transgenic plants.

**Résumé :** Les applications récentes au domaine végétal des techniques de la biologie moléculaire et de la biotechnologie pour l'étude des interactions hôtes-pathogènes ont permis d'identifier et de cloner plusieurs gènes impliqués dans les réponses de défense des plantes par suite d'une infection par un agent pathogène. Ceux-ci comprennent : des gènes qui codent pour des protéines, des peptides ou des composés antimicrobiens qui ont une toxicité directe ou réduisent la croissance in situ des agents pathogènes; des produits géniques qui inhibent directement des produits de virulence des agents pathogènes ou stimulent les gènes de défense structurale des plantes, qui activent directement ou indirectement les réponses générales de défense des plantes et des gènes de résistance impliqués dans la réaction d'hypersensibilité et dans les interactions avec les facteurs d'avirulence. L'introduction et l'expression de ces gènes, ainsi que celles de gènes antimicrobiens de source non végétale, dans une gamme d'espèces végétales transgéniques ont montré que le développement des champignons pathogènes peut être significativement réduit. L'ampleur de la réduction de la maladie dépend de la stratégie utilisée aussi bien que des caractéristiques du champignon pathogène; une répression complète de la maladie n'a pas encore été atteinte. La manipulation des niveaux d'acide salicylique, d'éthylène et de cytokinines dans des plantes transgéniques a fourni quelques résultats intéressants concernant la tolérance ou la sensibilité accrues à la maladie. Les interactions complexes entre les produits géniques exprimés, les espèces végétales et les champignons pathogènes laissent voir que la réponse des plantes transgéniques ne peut être parfaitement prédite. Les combinaisons de produits géniques de défense ont été beaucoup plus prometteuses.

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que les introductions d'un seul transgène pour lutter contre les maladies. L'emploi de promoteurs spécifiques à un tissu ou inducibles par les agents pathogènes et le génie appliqué à l'expression de gènes de résistance, de peptides synthétiques antimicrobiens et de molécules inductrices qui stimulent les réponses de défense ont le potentiel pour fournir commercialement, dans un futur pas si lointain, un moyen pratique pour résister à un large spectre de maladies. Les problèmes et les défis qui devront être surmontés avant d'arriver à une utilisation à grande échelle de ces plantes transgéniques sont mis en évidence.

**Mots clés :** protéines antifongiques, peptides antimicrobiens, biotechnologie, éliciteurs, réaction d'hypersensibilité, protéines reliées à la pathogénèse, phytoalexines, gènes de résistance, plantes transgéniques.

## Introduction

One of the challenges facing breeders during the development of improved crop cultivars for agricultural use is the incorporation of resistance to diseases. Since domestication of plants for human use began, diseases have caused major yield losses and have impacted the well-being of humans worldwide (Agrios 1997). The incorporation of disease resistance genes into plants has been successfully achieved using conventional breeding methods, which involve selection and evaluation of large progeny populations derived from crosses made between resistant and susceptible parents and subsequent screening under disease conducive conditions. Virtually all agricultural crop cultivars in use today have some form of genetic resistance incorporated, generally against a number of diseases. This may involve single or multiple genes that are characterized as having recessive or dominant effects (Crute and Pink 1996). Without the incorporation of these resistance genes, crop productivity and yield would be substantially reduced (Agrios 1997).

With the beginning of the molecular era of plant biology in the early 1980's, a major area of research has been to identify, clone, and characterize various genes involved in disease resistance. As a result, many intriguing mechanisms, which plants have evolved to respond to pathogen infection, have been identified over the past 10 years, and remarkable progress has been made toward elucidating the multitude of genes that are involved in these responses. The identification of these genes has made it possible to subsequently evaluate their specific roles and importance in disease response pathways using transgenic plants developed with genetic engineering techniques (Fig. 1).

In this paper, I will review advances made in utilizing a broad range of cloned genes (from both plant and nonplant sources) to enhance disease resistance against fungal pathogens in transgenic plants and address future challenges and prospects. Several other reviews on the subject of genetic engineering for fungal disease resistance provide related information on this topic (Shah 1997; Swords et al. 1997; Bushnell et al. 1998; Evans and Greenland 1998; Honée 1999; Melchers and Stuijver 2000; Rommens and Kishore 2000). The approaches that have been taken by researchers can be grouped into five general categories (see Table 1):

(1) The expression of gene products that are directly toxic to pathogens or that reduce their growth. These include pathogenesis-related proteins (PR proteins) such as hydrolytic enzymes (chitinases, glucanases), antifungal proteins (osmotin- and thaumatin-like), antimicrobial peptides (thionins, defensins, lectins), ribosome-inactivating proteins (RIP), and phytoalexins.

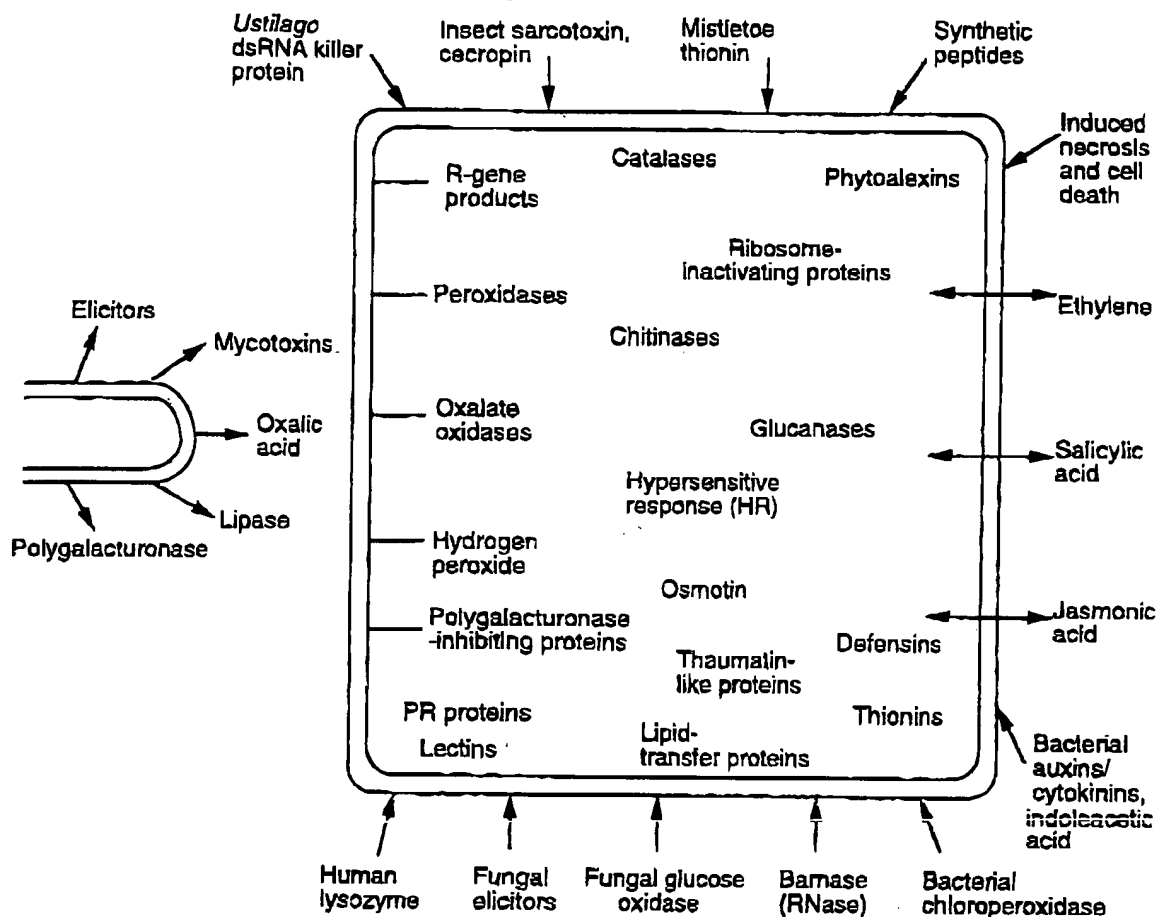
- (2) The expression of gene products that destroy or neutralize a component of the pathogen arsenal such as polygalacturonase, oxalic acid, and lipase.
- (3) The expression of gene products that can potentially enhance the structural defenses in the plant. These include elevated levels of peroxidase and lignin.
- (4) The expression of gene products releasing signals that can regulate plant defenses. This includes the production of specific elicitors, hydrogen peroxide ( $H_2O_2$ ), salicylic acid (SA), and ethylene ( $C_2H_4$ ).
- (5) The expression of resistance gene (R) products involved in the hypersensitive response (HR) and in interactions with avirulence (Avr) factors.

The selection of genes to genetically engineer into plants to protect against fungal diseases has been based, in part, on evaluation of the toxicity of the gene product to fungal growth or development in vitro, and to the prominence of the particular gene(s) in a disease resistance response pathway. Many gene products belong to the group of PR proteins (Neuhaus 1999; Van Loon and Van Strien 1999), while others are involved in phytoalexin biosynthetic pathways and in enhancing plant structural defenses. Although some of these gene products may normally be expressed relatively late in the response pathway, e.g. after 48 h, the rationale for developing transgenic plants was to achieve early and high expression (overexpression) of these proteins, usually constitutively throughout most of the plant. In other instances, enhanced levels of protein expression were reasoned to provide a greater inhibitory effect on fungal development than lower naturally occurring or induced levels in the plant. Other genes were selected in genetic engineering efforts for their ability to induce an array of naturally occurring defense mechanisms in the plant. More recently, the cloning of R genes has precipitated interest in utilizing these genes to provide broad-spectrum disease resistance. Some genetic engineering approaches have been based on novel approaches of introducing genes from double-stranded RNA entities from viruses found in fungi (Clausen et al. 2000) and genes of lysozymes cloned from human tissues (Nakajima et al. 1997; Takaichi and Oeda 2000) and from a range of microbes (Lorito and Scala 1999).

## Hydrolytic enzymes

The most widely used approach has been to overexpress chitinases and glucanases, which belong to the group of PR proteins (Neuhaus 1999) and have been shown to exhibit antifungal activity in vitro (Bolter 1993; Yun et al. 1997). Since chitins and glucans comprise major components of

Fig. 1. Transgenic plants with enhanced disease resistance have been engineered to express gene products to counterattack fungal virulence products (from hypha on left), enhanced expression of plant-derived gene products (inside of cell) or gene products from nonplant sources (outside of cell). The results from these experiments are summarized in Table 1.



the cell wall in many groups of fungi, the overexpression of these enzymes in plant cells is postulated to cause the hyphae to lyse and thereby reduce fungal growth (Mauch and Staehelin 1989). The specific roles of these hydrolases in resistance to disease have been difficult to prove in nontransgenic plants, since the enzymes are frequently encountered in both resistant and susceptible tissues, and their expression can also be induced by environmental triggers and plant senescence (Punja and Zhang 1993). However, following expression of different types of chitinases in a range of transgenic plant species, the rate of lesion development and the overall size and number of lesions were reduced upon challenge with many fungal pathogens (Table 1), including those with a broad host range, such as *Botrytis cinerea* and *Rhizoctonia solani*. However, chitinase expression was ineffective against other pathogens, such as *Cercospora nicotianae*, *Colletotrichum lagenarium*, and *Pythium* spp., indicating that differences exist in sensitivity of fungi to chitinase. The characteristics of chitinases from different sources can vary, e.g. in substrate binding specificity, pH optimum, and localization in the cell, and this can lead to differences in antifungal activity (Sela-Buurlage et al. 1993), highlighting the importance of appropriate selection of the gene to be used against a targeted pathogen or

group of pathogens. While the results from these efforts have not been spectacular in terms of the level of disease control, they demonstrate that the rate of disease progress and overall disease severity can be significantly reduced. A few transgenic crop species expressing chitinases have been evaluated in field trials and it was demonstrated that disease incidence was reduced (Howie et al. 1994; Grison et al. 1996; Melchers and Stuijver 2000).

There are fewer examples of the expression of glucanases in transgenic plants (Table 1) but the results have generally been similar to that for chitinase expression. The combined expression of chitinase and glucanase in transgenic carrot, tomato, and tobacco was much more effective in preventing development of disease due to a number of pathogens than either one alone (Jongedijk et al. 1995; Van den Elzen et al. 1993; Zhu et al. 1994), confirming the synergistic activity of these two enzymes reported from in vitro studies (Sela-Buurlage et al. 1993; Van den Elzen et al. 1993; Melchers and Stuijver 2000). As a general rule, the deployment of genetic engineering approaches that involve the expression of two or more antifungal gene products in a specific crop should provide more effective and broad-spectrum disease control than the single-gene strategy (Lamb et al. 1992; Cornelissen and Melchers 1993; Strittmatter and Wegner

Table 1. Plant species genetically engineered to enhance resistance to fungal diseases (1991–2001).

Strategy used and plant species engineered	Expressed gene product	Effect on disease development	Reference
<b>Expression of hydrolytic enzymes</b>			
Alfalfa ( <i>Medicago sativa</i> L.)	Alfalfa glucanase	Reduced symptom development due to <i>Phytophthora megasperma</i> ; no effect on <i>Stemphylium alfalfae</i>	Masoud et al. (1996)
American ginseng ( <i>Panax quinquefolius</i> L.)	Rice chitinase	Not tested	W.P. Chen and Z.K. Punja (unpublished data)
Apple ( <i>Malus domestica</i> Auth.)	<i>Trichoderma harzianum</i> endochitinase	Reduced lesion number and lesion area due to <i>Venturia inaequalis</i>	Bolar et al. (2000); Wong et al. (1999)
Barley ( <i>Hordeum vulgare</i> L.)	<i>Trichoderma endo</i> -1, 4- $\beta$ -glucanase	Not tested	Nuutila et al. (1999)
Canola ( <i>Brassica napus</i> L.)	Bean chitinase	Reduced rate and total seedling mortality due to <i>Rhizoctonia solani</i>	Broglie et al. (1991)
	Tomato chitinase	Lower percentage of diseased plants due to <i>Cylindrosporium concentricum</i> and <i>Sclerotinia sclerotiorum</i>	Grisson et al. (1996)
Carrot ( <i>Daucus carota</i> L.)	Tobacco chitinase	Reduced rate and final incidence of disease due to <i>Botrytis cinerea</i> , <i>Rhizoctonia solani</i> , and <i>Sclerotium rolfsii</i> ; no effect on <i>Thielaviopsis basicola</i> and <i>Alternaria radicina</i>	Punja and Raharjo (1996)
Chrysanthemum ( <i>Dendranthema grandiflorum</i> ) (Ramat.) Kitamura	Rice chitinase	Reduced lesion development due to <i>Botrytis cinerea</i>	Takatsu et al. (1999)
Cucumber ( <i>Cucumis sativus</i> L.)	Perunia and tobacco chitinases	No effect on disease development due to <i>Colletotrichum lagenarium</i> and <i>Rhizoctonia solani</i>	Punja and Raharjo (1996)
	Rice chitinase	Reduced lesion development due to <i>Botrytis cinerea</i>	Tabei et al. (1998)
Grape ( <i>Vitis vinifera</i> L.)	Rice chitinase	Reduced development of <i>Uncinula necator</i> and fewer lesions due to <i>Elisinoe unipelina</i>	Yamamoto et al. (2000)
	<i>Trichoderma harzianum</i> endochitinase	Reduction of <i>Botrytis cinerea</i> development in preliminary tests	Kikkert et al. (2000)
Peanut ( <i>Arachis hypogaea</i> )	Tobacco chitinase	Delayed lesion development and smaller lesion size due to <i>Cercospora arachidicola</i>	Rohini and Rao (2001)
Potato ( <i>Solanum tuberosum</i> L.)	<i>Trichoderma harzianum</i> endochitinase	Lower lesion numbers and size due to <i>Alternaria solani</i> ; reduced mortality due to <i>Rhizoctonia solani</i>	Lorito et al. (1998)
Rice ( <i>Oryza sativa</i> )	Rice chitinase	Delayed onset and reduced severity of disease symptoms due to <i>Magnaporthe grisea</i>	Nishizawa et al. (1999)
	Rice chitinase	Fewer numbers of lesions and smaller size due to <i>Rhizoctonia solani</i>	Lin et al. (1995); Datta et al. (2000, 2001)
Rose ( <i>Rosa hybrida</i> L.)	Rice chitinase	Reduced lesion diameter due to black spot ( <i>Diplocarpon rosae</i> )	Marchant et al. (1998)
Strawberry ( <i>Fragaria xananassa</i> Duch.)	Rice chitinase	Reduced development of powdery mildew ( <i>Sphaerotheca humuli</i> )	Asao et al. (1997)
Tobacco ( <i>Nicotiana benthamiana</i> L.)	Sugarbeet chitinase	No effect on <i>Cercospora nicotianae</i>	Nielsen et al. (1993)
Tobacco ( <i>Nicotiana sylvestris</i> L.)	Tobacco chitinase	No effect on <i>Cercospora nicotianae</i>	Neuhaus et al. (1991)
Tobacco ( <i>Nicotiana tabacum</i> L.)	Tobacco chitinase	Reduced colonization by <i>Rhizoctonia solani</i>	Vierheilig et al. (1993)
	Bean chitinase	Lower seedling mortality due to <i>Rhizoctonia solani</i> ; no effect on <i>Pythium aphanidermatum</i>	Broglie et al. (1991, 1993)
Tobacco ( <i>N. tabacum</i> L.)	Peanut chitinase	Not tested	Kellmann et al. (1996)

Table 1 (continued).

Strategy used and plant species engineered	Expressed gene product	Effect on disease development	Reference
	<i>Serratia marcescens</i> chitinase	Reduced disease incidence due to <i>Rhizoctonia solani</i> on seedlings; no effect on <i>Pythium ultimum</i>	Howie et al. (1994)
	<i>Serratia marcescens</i> chitinase	Reduced development of <i>Rhizoctonia solani</i>	Jach et al. (1992)
	<i>Rhizopus oligosporus</i> chitinase	Reduced rate of development and size of lesions on leaves due to <i>Borytis cinerea</i> and <i>Sclerotinia sclerotiorum</i>	Terakawa et al. (1997)
	<i>Streptomyces</i> chitosanase	Not tested	El Quakfaoui et al. (1995)
	<i>Trichoderma harzianum</i> endochitinase	Reduced symptoms due to <i>Alternaria alternata</i> , <i>Borytis cinerea</i> , and <i>Rhizoctonia solani</i>	Lorito et al. (1998)
	Baculovirus chitinase	Reduced lesion development due to brown spot ( <i>Alternaria alternata</i> )	Shi et al. (2000)
	Soybean glucanase	Reduced development of <i>Phytophthora parasitica</i> and <i>Alternaria alternata</i>	Yoshikawa et al. (1993)
	Tobacco glucanase	Reduced disease symptoms due to <i>Phytophthora parasitica</i> and <i>Peronospora tabacina</i>	Lusso and Kuc (1996)
	<i>Acidothermus cellulolyticus</i> endoglucanase	Not tested	Dai et al. (2000)
Tomato ( <i>Lycopersicon esculentum</i> Mill.)	Wild tomato ( <i>Lycopersicon chilense</i> ) chitinase	Reduced development of <i>Verticillium dahliae</i> races 1 and 2	Tabaeizadeh et al. (1999)
Wheat ( <i>Triticum aestivum</i> L.)	Barley chitinase	Reduced development of colonies of <i>Blumeria graminis</i> f. sp. <i>tritici</i>	Bliffeld et al. (1999)
	Barley chitinase	Reduced development of colonies of <i>Blumeria graminis</i> and <i>Puccinia recondita</i>	Oldach et al. (2001)
<b>Expression of pathogenesis-related (PR) proteins</b>			
Canola ( <i>B. napus</i> )	Pea chitinase, PR-10.1 gene	No effect on <i>Leptosphaeria maculans</i>	Wang et al. (1999)
	Pea defense response gene, defensin	Reduced infection and development of <i>Leptosphaeria maculans</i>	Wang et al. (1999)
Carrot ( <i>D. carota</i> )	Rice TLP	Reduced rate and final disease incidence due to <i>Borytis cinerea</i> and <i>Sclerotinia sclerotiorum</i>	W.P. Chen and Z.K. Punja (unpublished data)
Potato ( <i>Solanum commersonii</i> Dun.)	Potato osmotin-like protein	Enhanced tolerance to infection by <i>Phytophthora infestans</i>	Zhu et al. (1996)
Potato ( <i>S. tuberosum</i> )	Tobacco osmotin	Delayed onset and rate of disease due to <i>Phytophthora infestans</i>	Liu et al. (1994)
	Pea PR-10 gene	Reduced development of <i>Verticillium dahliae</i>	Chang et al. (1993)
	Potato defense response gene <i>STH-2</i>	No effect against <i>Phytophthora infestans</i>	Constabel et al. (1993)
Rice ( <i>O. sativa</i> )	Rice <i>Rir1b</i> defense gene	Fewer lesions due to <i>Magnaporthe grisea</i>	Schaffrath et al. (2000)
	Rice TLP	Reduced lesion development due to <i>Rhizoctonia solani</i>	Datta et al. (1999)
Tobacco ( <i>N. tabacum</i> )	Tobacco PR-1a	Reduced rate and final disease due to <i>Peronospora tabacina</i> and <i>Phytophthora parasitica</i>	Alexander et al. (1993)
	Tobacco osmotin	No effect on <i>Phytophthora parasitica</i> var. <i>nicotianae</i>	Liu et al. (1994)



Table 1 (continued).

Strategy used and plant species engineered	Expressed gene product	Effect on disease development	Reference
Wheat ( <i>T. aestivum</i> )	<i>Aspergillus giganteus</i> antifungal protein	Reduced development of colonies of <i>Blumeria graminis</i> and <i>Puccinia recondita</i>	Oldach et al. (2001)
	Rice TLP	Delayed development of <i>Fusarium graminearum</i>	Chen et al. (1999)
<b>Expression of antimicrobial proteins, peptides, or compounds</b>			
<i>Arabidopsis thaliana</i> L.	<i>Arabidopsis</i> thionin	Reduced development and colonization by <i>Fusarium oxysporum</i>	Epple et al. (1997)
	Mistletoe thionin viscotoxin	Reduced infection and development of <i>Plasmodiophora brassicae</i>	Holtorf et al. (1998)
Carrot ( <i>D. carota</i> )	Human lysozyme	Enhanced resistance to <i>Erysiphe heraclei</i> and <i>Alternaria dauci</i>	Takaichi and Oeda (2000)
Geranium ( <i>Pelargonium</i> sp.)	Onion antimicrobial protein	Reduced development and sporulation of <i>Botrytis cinerea</i>	Bi et al. (1999)
Potato ( <i>S. tuberosum</i> )	Alfalfa defensin	Enhanced resistance to <i>Verticillium dahliae</i>	Gao et al. (2000)
	<i>Bacillus amyloliquefaciens</i> barnase (RNase)	Delayed sporulation and reduced sporangia production by <i>Phytophthora infestans</i>	Strittmatter et al. (1995)
	Synthetic cationic peptide chimera	Reduced development of <i>Fusarium solani</i> and <i>Phytophthora cactorum</i>	Osusky et al. (2000)
	Human lactoferrin	Not tested	Chong and Langridge (2000)
Rice ( <i>O. sativa</i> )	Maize RIP	No effect on <i>Magnaporthe grisea</i> or <i>Rhizoctonia solani</i>	Kim et al. (1999)
Tobacco ( <i>N. tabacum</i> )	<i>Amaranthus</i> hevein-type peptide, <i>Mirabilis</i> knottin-type peptide	No effect on <i>Alternaria longipes</i> or <i>Botrytis cinerea</i>	De Bolle et al. (1996)
	Radish defensin	Reduced infection and lesion size due to <i>Alternaria longipes</i>	Terras et al. (1995)
	Barley RIP	Reduced incidence and severity of <i>Rhizoctonia solani</i>	Logemann et al. (1992)
	Maize RIP	Lower damage due to <i>Rhizoctonia solani</i>	Maddaloni et al. (1997)
	Pokeweed antiviral protein	Lower rate of infection and mortality due to <i>Rhizoctonia solani</i>	Wang et al. (1998); Zoubenko et al. (1997)
	Sarcotoxin peptide from <i>Sarcophaga peregrina</i>	Enhanced seedling survival following inoculation with <i>Rhizoctonia solani</i> , <i>Pythium aphanidermatum</i> , and <i>Phytophthora nicotianae</i>	Mitsuhara et al. (2000)
	Stinging nettle ( <i>Urtica dioica</i> L.) isolectin	Not tested	Does et al. (1999)
	Antifungal (killing) protein from virus infecting <i>Ustilago maydis</i> (dsRNA)	Not tested	Park et al. (1996)
	Chloroperoxidase from <i>Pseudomonas pyrocinia</i>	Reduced lesion development by <i>Colletotrichum destructivum</i>	Rajasekaran et al. (2000)
	Synthetic antimicrobial peptide	Reduced lesion size due to <i>Colletotrichum destructivum</i>	Cary et al. (2000)
	Synthetic magainin-type peptide	Reduced lesion size and sporulation due to <i>Peronospora tabacina</i>	Li et al. (2001)
	Human lysozyme	Reduced colony size and conidial production by <i>Erysiphe cichoracearum</i>	Nakajima et al. (1997)
Tomato ( <i>L. esculentum</i> )	Radish defensin	Reduced number and size of lesions due to <i>Alternaria solani</i>	Parashina et al. (2000)

Table 1 (continued).

Strategy used and plant species engineered	Expressed gene product	Effect on disease development	Reference
Wheat ( <i>T. aestivum</i> )	Barley RIP	Slightly reduced development of <i>Blumeria graminis</i>	Bieri et al. (2000)
	Antifungal (killing) protein from virus infecting <i>Ustilago maydis</i> (dsRNA)	Inhibition of <i>Ustilago maydis</i> and <i>Tilletia tritici</i> development on seeds	Clausen et al. (2000)
<b>Expression of phytoalexins</b>			
Alfalfa ( <i>M. sativa</i> )	Alfalfa isoflavone O-methyltransferase	Reduced lesion size due to <i>Phoma medicaginis</i>	He and Dixon (2000)
	Peanut resveratrol synthase	Reduced lesion size and sporulation of <i>Phoma medicaginis</i>	Hipskind and Paiva (2000)
Barley ( <i>H. vulgare</i> )	Grape stilbene (resveratrol) synthase	Reduced colonization by <i>Botrytis cinerea</i>	Leckband and Lörz (1998)
Rice ( <i>O. sativa</i> )	Grape stilbene (resveratrol) synthase	Reduced lesion development due to <i>Pyricularia oryzae</i>	Stark-Lorenzen et al. (1997)
Tobacco ( <i>N. tabacum</i> )	<i>Fusarium</i> trichodiene synthase	Not tested	Zook et al. (1996)
	Grape stilbene (resveratrol) synthase	Reduced colonization by <i>Botrytis cinerea</i>	Hain et al. (1993)
Tomato ( <i>L. esculentum</i> )	Grape stilbene (resveratrol) synthase	Reduced lesion development by <i>Phytophthora infestans</i> ; no effect on <i>Alternaria solani</i> or <i>Botrytis cinerea</i>	Thomzik et al. (1997)
Wheat ( <i>T. aestivum</i> )	Grape stilbene (resveratrol) synthase	Not tested	Fettig and Hess (1999)
<b>Inhibition of pathogen virulence products</b>			
Canola ( <i>B. napus</i> )	Barley oxalate oxidase	Not tested	Thompson et al. (1995)
Poplar ( <i>Populus xeuramericana</i> Auth.)	Wheat oxalate oxidase	Delayed development of <i>Septoria musiva</i>	Liang et al. (2001)
Tobacco ( <i>N. tabacum</i> )	<i>Fusarium</i> trichothecene-degrading enzyme	Not tested	Muhitch et al. (2000)
	Wheat oxalate oxidase (germin)	Not tested	Berna and Bernier (1997)
Tomato ( <i>L. esculentum</i> )	Bean polygalacturonase inhibiting protein	No effect on disease due to <i>Fusarium oxysporum</i> , <i>Botrytis cinerea</i> , and <i>Alternaria solani</i>	Desiderio et al. (1997)
	Pear polygalacturonase inhibiting protein	Reduced rate of development of <i>Botrytis cinerea</i>	Powell et al. (2000)
	<i>Collybia velutipes</i> oxalate decarboxylase	Enhanced resistance to <i>Sclerotinia sclerotiorum</i>	Kesarwani et al. (2000)
<b>Alteration of structural components</b>			
Potato ( <i>S. tuberosum</i> )	Cucumber peroxidase	No effect on disease due to <i>Fusarium sambucinum</i> and <i>Phytophthora infestans</i>	Ray et al. (1998)
Tomato ( <i>L. esculentum</i> )	Tobacco anionic peroxidase	No effect on disease due to <i>Fusarium oxysporum</i> and <i>Verticillium dahliae</i>	Lagrimini et al. (1993)
Wheat ( <i>T. aestivum</i> )	Wheat germin (no oxalate oxidase activity)	Reduced penetration by <i>Blumeria graminis</i> into epidermal cells	Schweizer et al. (1999)
<b>Regulation of plant defense responses</b>			
<i>A. thaliana</i>	<i>Arabidopsis</i> NPR1 protein	Reduced infection and growth of <i>Peronospora parasitica</i>	Cao et al. (1998)

Table 1 (concluded).

Strategy used and plant species engineered	Expressed gene product	Effect on disease development	Reference
Cotton ( <i>Gossypium hirsutum</i> L.), tobacco ( <i>N. tabacum</i> )	<i>Talaromyces flavus</i> glucose oxidase	Enhanced protection against <i>Rhizoctonia solani</i> and <i>Verticillium dahliae</i> ; no effect on <i>Fusarium oxysporum</i>	Murray et al. (1999)
Potato ( <i>S. tuberosum</i> )	<i>Aspergillus niger</i> glucose oxidase	Delayed lesion development due to <i>Phytophthora infestans</i> ; reduced disease development due to <i>Alternaria solani</i> and <i>Verticillium dahliae</i>	Wu et al. (1995, 1997)
	Tobacco catalase	Reduced lesion size due to <i>Phytophthora infestans</i>	Yu et al. (1999)
	Bacterial salicylate hydroxylase	No effect on <i>Phytophthora infestans</i>	Yu et al. (1997)
Tobacco ( <i>N. tabacum</i> ), <i>A. thaliana</i>	Bacterial salicylate hydroxylase	Enhanced susceptibility to <i>Phytophthora parasitica</i> , <i>Cercospora nicotianae</i> , and <i>Peronospora parasitica</i>	Delaney et al. (1994); Donofrio and Delaney (2001)
Tobacco ( <i>N. tabacum</i> )	Bacterial enzymes generating salicylic acid	Enhanced resistance to <i>Oidium lycopersicon</i>	Verberne et al. (2000)
	<i>Arabidopsis</i> ethylene-insensitivity gene	Enhanced susceptibility to <i>Pythium sylvaticum</i>	Knoester et al. (1998)
	<i>Phytophthora cryptogea</i> elicitor ( $\beta$ -cryptogein)	Reduced infection by <i>Phytophthora parasitica</i>	Tepfer et al. (1998)
	<i>Phytophthora cryptogea</i> elicitor (cryptogein)	Enhanced resistance to <i>Phytophthora parasitica</i> , <i>Thielaviopsis basicola</i> , <i>Borreria cinerea</i> , and <i>Erysiphe cichoracearum</i>	Keller et al. (1999)
<b>Expression of combined gene products</b>			
Carrot ( <i>D. carota</i> )	Tobacco chitinase + $\beta$ -1,3-glucanase, osmotin	Enhanced resistance to <i>Alternaria dauci</i> , <i>Alternaria radicina</i> , <i>Cercospora carotae</i> , and <i>Erysiphe heraclei</i>	Melchers and Stuiver (2000)
Tobacco ( <i>N. tabacum</i> )	Barley chitinase + $\beta$ -1,3-glucanase, or chitinase + RIP	Reduced disease severity due to <i>Rhizoctonia solani</i>	Jach et al. (1995)
	Rice chitinase + alfalfa glucanase	Reduced rate of lesion development and fewer lesions due to <i>Cercospora nicotianae</i>	Zhu et al. (1994)
Tomato ( <i>L. esculentum</i> )	Tobacco chitinase + $\beta$ -1,3-glucanase	Reduced disease severity due to <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	Jongedijk et al. (1995); Van den Elzen et al. (1993)

Note: dsRNA, double-stranded RNA; RIP, ribosome-inactivating protein; TLP, thaumatin-like protein.

1993; Jach et al. 1995; Shah 1997; Evans and Greenland 1998; Salmeron and Vernooij 1998; Melchers and Stuiver 2000).

### Pathogenesis-related proteins

Other PR proteins that exhibit antifungal activity, including osmotin- and thaumatin-like proteins (TLP), and some uncharacterized PR proteins have been engineered into crop plants (Table 1). Osmotin is a basic 24-kDa protein belonging to the PR-5 family whose members have a high degree of homology to the sweet-tasting protein thaumatin from *Thaumatococcus danielli* and are produced in plants under different stress conditions (Zhu et al. 1995). The PR-5 proteins induce fungal cell leakiness, presumably through a specific interaction with the plasma membrane that results in the formation of transmembrane pores (Kitajima and Sato 1999). Osmotin has been shown to have antifungal activity in vitro (Woloshuk et al. 1991; Melchers et al. 1993; Liu et al. 1994) and, when tested in combination with

chitinase and  $\beta$ -1,3-glucanase, showed enhanced lytic activity (Lorito et al. 1996). When expressed in transgenic potato, osmotin was shown to delay expression of disease symptoms caused by *Phytophthora infestans* (Table 1). Thaumatin-like proteins are also expressed in plants in response to a range of stress conditions and were demonstrated to have antifungal activity in vitro (Malehorn et al. 1994; Koiwa et al. 1997). Expression of TLP in transgenic plants was reported to delay disease development due to several pathogens, including *Borreria*, *Fusarium*, *Rhizoctonia*, and *Sclerotinia* (Table 1). Combinations of PR-5 protein expression with chitinases or glucanases in transgenic plants have not been reported and it is anticipated that the level of disease reduction achieved would be significantly higher.

### Antimicrobial proteins, peptides, and other compounds

Defensins and thionins are low molecular mass (around 5 kDa) cysteine-rich peptides (45–54 amino acids in length)

found in monocotyledonous and dicotyledonous plant species, which were initially derived from seeds and have antimicrobial activity (Bohlmann 1994; Broekaert et al. 1995; Evans and Greenland 1998). Viscotoxin from mistletoe (*Viscum album*) is a thionin. It was proposed that these peptides play a role in protecting seeds from infection by pathogens (Broekaert et al. 1997). Defensins are also found in insects and mammals, where they play an important role in curtailing or limiting microbial attack (Rao 1995). These peptides may exert antifungal activity by altering fungal membrane permeability and (or) inhibiting macromolecule biosynthesis, and thionins may be toxic to plant and animal cell cultures as well (Broekaert et al. 1997). The over-expression of defensins and thionins in transgenic plants was demonstrated to reduce development of several different pathogens, including *Alternaria*, *Fusarium*, and *Plasmidiophora* (Table 1), and provided resistance to *Verticillium* on potato under field conditions (Gao et al. 2000).

Chitin-binding peptides (hevein- and knottin-types) are 36–40 residues in length and have been recovered from the seeds of some plant species. They contain cysteine residues and were demonstrated to have antifungal activity in vitro (Broekaert et al. 1997). However, expression of *Amaranthus* hevein-type peptide and *Mirabilis* knottin-type peptide in transgenic tobacco did not enhance tolerance to *Alternaria longipes* or *Botrytis cinerea* (De Bolle et al. 1996). It was postulated that the presence of cations, particularly  $\text{Ca}^{2+}$ , may have inhibited the activity of these peptides in vivo. Modifications to amino acid sequences of peptides may enhance the antifungal activity (Evans and Greenland 1998).

Ribosome-inactivating proteins are plant enzymes that have 28 S rRNA N-glycosidase activity, which depending on their specificity, can inactivate conspecific or foreign ribosomes, thereby shutting down protein synthesis. The most common cytosolic type I RIP from the endosperm of cereal grains do not act on plant ribosomes but can affect foreign ribosomes, such as those of fungi (Stirpe et al. 1992; Hartley et al. 1996). Expression of barley seed RIP reduced development of *Rhizoctonia solani* in transgenic tobacco (Logemann et al. 1992) but had little effect on *Blumeria graminis* in transgenic wheat (Bieri et al. 2000). In the latter study, the RIP was targeted to the apoplastic space and may have had less activity against development of the intracellular haustoria of the mildew pathogen. It has been demonstrated that combined expression of chitinase and RIP in transgenic tobacco had a more inhibitory effect on *Rhizoctonia solani* development than the individual proteins (Jach et al. 1995). Therefore, dissolution of the fungal cell wall by hydrolytic enzymes should enhance the efficacy of antifungal proteins and peptides in transgenic plants. Human lysozyme has lytic activity against fungi and bacteria, and when expressed in transgenic carrot and tobacco, enhanced resistance to several pathogens, including *Erysiphe* and *Alternaria* (Nakajima et al. 1997; Takaichi and Oeda 2000). An antimicrobial protein with homology to lipid transfer protein was shown to reduce development of *Botrytis cinerea* when expressed in transgenic geranium (Bi et al. 1999).

Pokeweed (*Pyrola americana*) antiviral protein with type I RIP activity has been expressed in transgenic tobacco and shown to reduce development of *Rhizoctonia solani* (Wang et al. 1998). Because of some toxicity to plant cells,

nontoxic mutant proteins were derived and their expression in transgenic plants led to the activation of defense-related signalling pathways and PR-protein induction (e.g., chitinase and glucanase), which in turn enhanced plant resistance to infection by *Rhizoctonia solani* (Zoubenko et al. 1997). The induction of defense pathways in transgenic plants using other strategies will be discussed later.

Antimicrobial peptides have been synthesized in the laboratory to produce smaller (10–20 amino acids in length) molecules that have enhanced potency against fungi (Cary et al. 2000). In addition, a synthetic cationic peptide chimera (cecropin–melittin) with broad-spectrum antimicrobial activity has been produced (Osusky et al. 2000). When expressed in transgenic potato and tobacco, these synthetic peptides have provided enhanced resistance against a number of fungal pathogens, including *Colletotrichum*, *Fusarium*, and *Phytophthora* (Table 1). These peptides may demonstrate lytic activity against fungal hyphae, inhibit cell wall formation, and (or) enhance membrane leakage. The ability to create synthetic recombinant and combinatorial variants of peptides that can be rapidly screened in the laboratory could provide additional opportunities to engineer resistance to a range of pathogens simultaneously. Enhancement of the specific activities of antifungal enzymes or the creation of variants with broad activity using directed molecular evolution (DNA shuffling) has also been proposed as a method to enhance the efficacy of transgenic plants in the future (Lassner and Bedbrook 2001).

## Phytoalexins

These are low molecular mass secondary metabolites produced in a broad range of plant species, which were demonstrated to have antimicrobial activity and are induced by pathogen infection and elicitors (Hammerschmidt 1999; Grayer and Kokubun 2001). Phytoalexins are synthesized through complex biochemical pathways (Dixon et al. 1996), such as the shikimic acid pathway, and genetic manipulation of these pathways to suppress or enhance phytoalexin production has been difficult to achieve. Similar to the hydrolytic enzymes, it has not been easy to conclusively demonstrate the role played by phytoalexins in enhancing resistance to disease in many host–pathogen interactions. A mutant of *Arabidopsis* deficient in the production of the indole-type phytoalexin camalexin was shown to be more susceptible to infection by *Alternaria brassicicola* but not to *Botrytis cinerea* (Thomma et al. 1999b). Using transgenic plants, it has been possible to also show that the over-expression of genes encoding certain phytoalexins, such as *trans-resveratrol* and *medicarpin*, resulted in delayed development of disease and symptom production by a number of pathogens on several plant species (Table 1). These studies are encouraging in light of the difficulties of engineering the complex biochemical pathways leading to phytoalexin accumulation in plants (Dixon et al. 1996).

## Inhibition of pathogen virulence products

The plant cell wall acts as a barrier to penetration by fungal pathogens and numerous strategies have evolved among plant pathogens to overcome this (Walton 1994). These include se-

cretion of a range of plant cell wall degrading enzymes (depolymerases) and the production of toxins such as oxalic acid by fungal pathogens. Several strategies to engineer resistance against fungal infection have targeted the inactivation of these pathogen virulence products. Polygalacturonase-inhibiting proteins (PGIP) are glycoproteins present in the cell wall of many plants and that can inhibit the activity of fungal endopolygalacturonases (Powell et al. 1994; Desiderio et al. 1997). The expression of PGIP in transgenic plants led to contrasting results: in transgenic tomato expressing a bean PGIP, resistance to *Fusarium*, *Botrytis*, or *Alternaria* was not enhanced (Desiderio et al. 1997) while in transgenic tomato expressing a pear PGIP, colonization of leaves and fruits by *Botrytis* was reduced (Powell et al. 2000). In the former study, it was shown that PGIPs from bean differed in specificity to fungal polygalacturonase in vitro, and the PGIP-1 that had been selected for transformation was not inhibitory (Desiderio et al. 1997). Thus, appropriate in vitro screening of PGIPs would be required prior to undertaking transformation experiments. As with the PR proteins and antifungal compounds, disease development was reduced by PGIPs but not totally prevented in the transgenic plants.

Another developed strategy that could have potential to reduce pathogen infection is immunomodulation, the expression of genes encoding antibodies or antibody fragments in plants (plantibodies) that could bind to pathogen virulence products (De Jaeger et al. 2000; Schillberg et al. 2001). The antibodies can be expressed inter- or extracellularly and can bind to and inactivate enzymes, toxins, or other pathogen factors involved in disease development. Currently, there are no published reports on the expression of antifungal antibodies in transgenic plants that have led to a reduction in disease. However, it has been demonstrated that antilipase antibodies inhibited infection of tomato by *Botrytis cinerea*, when mixed with spore inoculum, by preventing fungal penetration through the cuticle (Comménil et al. 1998). Similarly, infection by *Colletotrichum gloeosporioides* on various fruits was inhibited using polyclonal antibodies that bound to fungal pectate lyase (Wattad et al. 1997). Genetic engineering of antibody expression in plants is extremely challenging technically and the applications to fungal disease control (immunization) have yet to be determined, although success against virus diseases has been reported (De Jaeger et al. 2000).

Production of phytotoxic metabolites, such as mycotoxins and oxalic acid, by fungal pathogens has been shown to facilitate infection of host tissues following cell death. Degradation of these compounds by enzymes expressed in transgenic plants could provide an opportunity to enhance resistance to disease. Expression of a trichothecene-degrading enzyme from *Fusarium sporotrichioides* in transgenic tobacco reduced plant tissue damage and enhanced seedling emergence in the presence of the trichothecene (Muhitch et al. 2000). The effect on pathogen development was not tested. Germin-like oxalate oxidases are stable glycoproteins first discovered in cereals, which are present during seed germination and are induced in response to fungal infection and abiotic stress (Dumas et al. 1995; Zhang et al. 1995; Berna and Bernier 1997). Their activity on the substrate oxalic acid results in the production of  $\text{CO}_2$  and  $\text{H}_2\text{O}_2$ ; the latter can induce defense responses in the plant

and enhance strengthening of cell walls (Brisson et al. 1994; Mehdy 1994). The expression of barley oxalate oxidase in oilseed rape enhanced tolerance to the phytotoxic effects of oxalic acid, although the effect on the target pathogen *Sclerotinia sclerotiorum* was not evaluated (Thompson et al. 1995). Expression of oxalate oxidase in transgenic hybrid poplar enhanced resistance to *Septoria*, while oxalate decarboxylase expression enhanced resistance of tomato to *Sclerotinia sclerotiorum* (Table 1). These reports indicate that the inactivation of specific pathogen virulence factors, such as toxins, by gene products expressed in transgenic plants has the potential to reduce development of specific fungal pathogens.

## Alteration of structural components

Lignification of plant cells around sites of infection or lesions has been reported to be a defense response of plants that can potentially slow down pathogen spread (Nicholson and Hammerschmidt 1992). The enzyme peroxidase is required for the final polymerization of phenolic derivatives into lignin and may also be involved in suberization or wound healing. A decrease in polyphenolic compounds, such as lignin, in potato tubers by redirection of tryptophan in transgenic plants through expression of tryptophan decarboxylase rendered tissues more susceptible to *Phytophthora infestans* (Yao et al. 1995), illustrating the role of phenolic compounds in defense. Reduction of phenylpropanoid metabolism through inhibition of phenylalanine ammonia-lyase activity in transgenic tobacco also rendered tissues more susceptible to *Cercospora nicotianae* (Maher et al. 1994). Overexpression of a cucumber peroxidase gene in transgenic potato, however, did not increase resistance of tissues to infection by *Fusarium* or *Phytophthora*, and lignin levels were not significantly affected, in spite of elevated peroxidase expression (Ray et al. 1998). It was suggested that peroxidase levels may not have been the limiting step for lignification or that the native peroxidase activity may have been cosuppressed. Overexpression of a tobacco anionic peroxidase gene in tomato did enhance lignin levels but resistance to fungal pathogens was not enhanced (Lagrimini et al. 1993). Lignin levels were also significantly higher following expression of the  $\text{H}_2\text{O}_2$ -generating enzyme glucose oxidase in transgenic potato (Wu et al. 1997) and by expression of the hormone indoleacetic acid (IAA) in transgenic tobacco (Sitbon et al. 1999). In the former case, tolerance to several fungal pathogens was enhanced (Table 1). Peroxidase overexpression in plants can, however, have negative effects on plant growth and development (Lagrimini et al. 1997), and the results to date indicate that this approach appears to hold less promise for enhancing disease resistance.

A reduction in large callose deposits surrounding haustoria of *Peronospora parasitica* infecting *Arabidopsis thaliana* was indirectly achieved in transgenic plants not accumulating SA by expression of the enzyme salicylate hydroxylase (Donofrio and Delaney 2001). These plants also had reduced expression of the *PR-1* gene and exhibited significantly enhanced susceptibility to the pathogen, suggesting that callose deposition during normal defense responses of the plant was influenced by the reduced levels of SA.

## Activation of plant defense responses

One activator of host defense responses are elicitor molecules from an invading pathogen. These can trigger a network of signalling pathways that coordinate the defense responses of the plant, including HR, PR protein, and phytoalexin production (Heath 2000; McDowell and Dangel 2000; Shirasu and Schulze-Lefert 2000). A gene encoding the elicitor cryptogein (a small basic protein, 98 amino acids in length) from the pathogen *Phytophthora cryptogea* was cloned and expressed in transgenic tobacco under control of a pathogen-inducible promoter (Keller et al. 1999). Challenge inoculation with a range of fungi induced the HR as well as several defense genes, and growth of the pathogens was concomitantly restricted (Table 1). Resistance to the pathogens was not complete, possibly because of the time needed for production of the transgenic elicitor following initial infection (Keller et al. 1999). Another elicitor, INF1, was shown to act as an Avr factor in the tobacco - *Phytophthora infestans* interaction and triggered the onset of the HR (Kamoun et al. 1998). Expression of the gene encoding the AVR9 peptide elicitor from *Cladosporium fulvum* in transgenic tomatoes containing the Cf-9 gene resulted in a necrotic defense response (Hammond-Kosack et al. 1994; Honée et al. 1995). The development of lesions resembling the HR induced through expression of a bacterial proton pump gene (bacterio-opsin) from *Halobacterium halobium* activated multiple defense systems in transgenic tobacco plants (Mittler et al. 1995) in the absence of pathogen challenge. In transgenic potato, expression of bacterio-opsin enhanced resistance to some pathogens but had no effect on others (Abad et al. 1997), while in poplar, there was no effect on disease development (Mohamed et al. 2001). Antisense inhibition of catalase, a  $H_2O_2$ -degrading enzyme, resulted in development of necrotic lesions and PR-protein accumulation (Takahashi et al. 1997). While these and other reports indicate that induction of the HR and necrosis, with the resulting activation of general defense pathways, could potentially result in broad-spectrum disease resistance (Bent 1996; Honée 1999; Melchers and Stuijver 2000), the use of such an approach would require tight regulation of the expressed phenotype, in addition to ensuring that no deleterious side effects, such as abnormal or suppressed growth, occurred on the transgenic plants. If successful, the activation of general defense responses in these transgenic plants would provide protection against viral and bacterial pathogens in addition to fungi.

Another activator of defense responses that has been engineered in transgenic plants is  $H_2O_2$  generated through expression of genes encoding for glucose oxidase (Table 1).  $H_2O_2$  has been shown to directly inhibit pathogen growth (Wu et al. 1995) and to induce PR proteins, SA, and ethylene (Wu et al. 1997; Chamnongpol et al. 1998), as well as phytoalexins (Mehdy 1994). It is produced during the early oxidative burst in plant cell response to infection (Baker and Orlandi 1995) and can trigger the HR (Levine et al. 1994; Tenhaken et al. 1995), strengthen cell walls (Brisson et al. 1994), and enhance lignin formation (Wu et al. 1997). Expression of elevated levels of  $H_2O_2$  in transgenic cotton, tobacco, and potato reduced disease development due to a number of different fungi, including *Rhizoctonia*, *Verti-*

*cillium*, *Phytophthora*, and *Alternaria* (Table 1); high levels can, however, be phytotoxic (Murray et al. 1999). In one study, necrotic lesions from the HR enhanced infection by the necrotrophic pathogen *Botrytis cinerea* (Govrin and Levine 2000). Therefore, the widespread induction of cell death in a transgenic plant to induce disease resistance has to be approached with caution.

Other activators of plant defense responses include signalling molecules such as SA, ethylene, and jasmonic acid (Yang et al. 1997; Dong 1998; Reymond and Farmer 1998; Dempsey et al. 1999). The roles of SA as a signal molecule for the activation of plant defense responses to pathogen infection and as an inducer of systemic acquired resistance (SAR) have been extensively studied (Ryals et al. 1996; Stichter et al. 1997; Dempsey et al. 1999; Métraux 2001). Using transgenic plants, evidence for the role of SA in defense response activation has been obtained. Plants expressing the SA-metabolizing enzyme salicylate hydroxylase, a bacterial protein that converts SA to the inactive form catechol, did not accumulate high levels of SA and had enhanced susceptibility to pathogen infection (Gaffney et al. 1993; Delaney et al. 1994; Donofrio and Delaney 2001) or had unaltered susceptibility (Yu et al. 1997). A mutant of *Arabidopsis* nonresponsive to induction of SAR showed enhanced susceptibility to fungal infection (Delaney et al. 1995; Donofrio and Delaney 2001). The overexpression of SA in transgenic tobacco was recently shown to enhance PR-protein production and provide resistance to fungal pathogens (Verberne et al. 2000). Expression of tobacco catalase, an enzyme with SA-binding activity, in transgenic potato enhanced defense gene expression leading to SAR and enhanced tolerance to *Phytophthora infestans* (Yu et al. 1999). Overexpression of the *NPR1* gene, which regulates the SA-mediated signal leading to SAR induction, in transgenic *Arabidopsis* increased the level of PR proteins during infection and enhanced resistance to *Peronospora parasitica* (Cao et al. 1998). It was postulated that synergistic interactions between PR proteins and products of other downstream defense-related genes provided the enhanced resistance. In addition, *NPR1* was only activated upon infection or by induction of SAR, avoiding potential side effects on plant growth from constitutive expression. These studies demonstrate that manipulation of SA levels in transgenic plants has the potential to lead to enhanced disease resistance by inducing PR-protein expression and other defense gene products.

Ethylene and jasmonic acid appear to be signals used in response of plants to necrotrophic pathogen attack (in contrast to biotrophic infection) and that work independently of, and possibly antagonistic to, SA-mediated responses (Dong 1998; Thomma et al. 1999a; McDowell and Dangel 2000; Lee et al. 2001). Mutant or transformed plants nonresponsive to either jasmonate or ethylene were found to be more susceptible to infection by root- and foliar-infecting fungi (Knoester et al. 1998; Staswick et al. 1998; Vijayan et al. 1998; Hoffmann et al. 1999; Thomma et al. 1999a), confirming a role for these signals in certain host-pathogen interactions. In contrast, ethylene-insensitive mutants may exhibit reduced disease symptoms, as described for *Fusarium oxysporum* on tomato (Lund et al. 1998). Genetic engineering efforts to alter ethylene or jasmonate pro-

duction in plants may, however, result in unpredictable effects on disease response, depending on the pathogen, as well as induce potential side effects in view of the multiple roles played by these signal molecules in plants (O'Donnell et al. 1996; Weiler 1997; Wilkinson et al. 1997).

Ethylene production and extracellular PR-protein expression were found to be induced by expression of cytokinins in transgenic tomato cells (Bertini et al. 1998). The engineering of hormone biosynthetic gene expression in transgenic plants has been accomplished (Hedden and Phillips 2000). Whether or not reduced or elevated levels of hormones, such as auxins, cytokinins, gibberellins, and jasmonate, can lead to the development of transgenic plants with enhanced disease resistance remains to be seen, given their broad range of physiological effects on plant development. Interestingly, inhibition of IAA production by antisense transformation of the nitrilase 1 gene in *Arabidopsis* reduced levels of IAA and development of root galls due to *Plasmodiophora brassicae* (Neuhaus et al. 2000). In contrast, overexpression of IAA in tobacco enhanced ethylene production and peroxidase activity and increased lignin content although the response to disease was not tested (Sitbon et al. 1999). Altered auxin or cytokinin expression has the potential to also affect mycorrhizal colonization of plant roots (Barker and Tagu 2000).

## Resistance genes

Resistance gene products may serve as receptors for pathogen Avr factors or recognize the Avr factor indirectly through a coreceptor (Staskawicz et al. 1995). This gene-for-gene interaction triggers one or more signal transduction pathways that in turn activate defense responses in the plant to prevent pathogen growth (Hammond-Kosack and Jones 1996; De Wit 1997). These defense responses include the development of the HR, expression of PR proteins, and accumulation of SA and can lead to the development of SAR (Ryals et al. 1996; Dempsey et al. 1999; Kombrink and Schmelzer 2001). Ethylene and jasmonic acid may also be involved in signalling the defense responses in the gene-for-gene interaction (Deikman 1997; Dong 1998). Efforts to clone an array of R genes involved in fungal disease resistance have met with some success (Bent 1996; Crute and Pink 1996; Baker et al. 1997; De Wit 1997; Hammond-Kosack and Jones 1997; Ellis and Jones 1998;). The R-gene products that have been cloned from tomato, tobacco, rice, flax, *Arabidopsis*, and several other plant species shared one or more similar motifs: a serine or threonine kinase domain, a nucleotide binding site, a leucine zipper, or a leucine-rich repeat region, all of which may contribute to recognition specificity (Shirasu and Schulze-Lefert 2000; Takken and Joosten 2000). The *Hml* R gene cloned from maize is an exception, as it encodes for a NADPH-dependent reductase that inactivates the potent toxin produced by race 1 strains of *Cochliobolus carbonum* (Johal and Briggs 1992). Many R genes belong to tightly linked multigene families, e.g. *Cf-4* to *Cf-9* encoding resistance to *Cladosporium fulvum* mold of tomato (Thomas et al. 1997).

There are several examples of the expression of R genes in transgenic plants. The overexpression of the *HRT* gene, which controls the HR to turnip crinkle virus in *Arabidopsis*,

did not confer enhanced resistance to *Peronospora tabacina* (Cooley et al. 2000). The authors proposed that multiple factors may be involved in determining the resistance response, or that the resistance may be HR-independent. Expression of the *Cf-9* gene, which confers resistance in tomato to races of *Cladosporium fulvum*, in transgenic tobacco and potato gave rise to the HR when challenged with AVR9 peptide (Hammond-Kosack et al. 1998), indicating that the *Cf-9* gene product was produced. However, for the disease resistance mediated by R gene – Avr factor to be fully expressed, several additional loci may be required (Hammond-Kosack and Jones 1996; Baker et al. 1997), in addition to elevated levels of SA (Delaney et al. 1994). Results to date suggest that the expression of cloned R genes in heterologous transgenic plants is unlikely by itself to enhance tolerance to fungal pathogens because of the complexity of the interacting signalling pathways. A combination of several interacting genes, similar to that for the antifungal proteins, will likely be required. An enhanced understanding of R-gene structure and function could, however, make it possible to modify functional domains in the future to tailor R genes for use in providing broad-spectrum resistance to diseases in transgenic plants (Bent 1996; Dempsey et al. 1998). Other potential approaches to the use of R genes for engineering disease resistance in plants are discussed by Rommens and Kishore (2000).

## Scientific challenges

Besides identifying and cloning potentially useful genes to engineer into plants, the development of transgenic plants with enhanced fungal disease resistance faces additional challenges. Depending on the plant species, transformation frequencies can be as low as 1–10%, and out of hundreds of confirmed transgenic lines, only a few may have appropriate transgene expression levels. Recent advances in plant transformation should provide new opportunities to overcome some of these difficulties (Gelvin 1998; Hansen and Wright 1999; Newell 2000). The positive relationship of high levels of PR proteins and antifungal compounds with enhanced disease resistance in plants has been documented in many but not all cases. However, as indicated previously, there are a number of examples where transgene products expressed at high levels induced plant cell damage or had other undesirable effects. These include the engineered expression of thionins, RIP, peroxidase,  $H_2O_2$ , elicitor molecules, and growth regulators. In most instances, constitutive promoters have been used to achieve high expression levels throughout most tissues of the plant. In crops affected by pathogens that colonize more than one type of organ, e.g. roots and leaves, this is advantageous. In instances where only specific tissues need to be protected, e.g. leaves, fruit, or seed, or where the antifungal compounds need to be expressed at certain targeted sites in the cell, specific promoters would need to be identified (Bushnell et al. 1998; Dahleen et al. 2001). Wound-inducible and pathogen-inducible promoters, which have advantages for engineering specific disease resistance against fungal pathogens by expressing antifungal compounds only at sites of infection or wounds, have also been described (Roby et al. 1990; Strittmatter et al. 1995; Keller et al. 1999). Targeting of the



engineered protein to the apoplastic space or to the vacuole has been achieved in numerous previous studies and may enhance the antifungal activity, depending on the mode of infection of the pathogen. Future research will require the fine tuning of engineered gene expression and establishment of the optimal expression levels and target site in the cell needed to prevent pathogen infection.

## Commercial development

Compared to the demonstrable scientific and economic success in engineering crop plants for resistance to herbicides, insect pests, and virus diseases (Shah et al. 1995), engineering of plants with enhanced disease resistance has lagged behind. Despite close to 100 published scientific reports (Table 1), of which about 30% are on tobacco used as a model system, only a few of the transgenic crops have been field-tested, and wide-scale deployment may not yet be realized for another 5–10 years. Development of transgenic plants with enhanced disease resistance is also being actively pursued in the private sector, and recent unpublished developments may not be included here. It remains to be demonstrated under field conditions to which level disease resistance is achieved and whether it is against a range of phytopathogens or only specific diseases. It is noteworthy that many of the successfully controlled pathogens in laboratory and greenhouse evaluations are those with a wide host range, such as *Rhizoctonia solani* and *Botrytis cinerea*, for which there are few available sources of resistance through conventional breeding in most crops. This is particularly true also for seedling-infecting pathogens, for which there are few examples of genetic resistance in the host. Therefore, genetic engineering of novel disease resistance traits in crop plants has the potential to provide control of devastating pathogens with reduced fungicide applications. Expression of an antifungal trait throughout the growing season, from seed to harvest, under prolonged disease-conducive conditions, can also provide significant advantages for disease management using this technology.

## Issues to be addressed

The current challenges and issues surrounding the acceptance of genetically modified foods will need to be addressed before crop plants engineered for increased resistance to fungal pathogens are successfully brought to market (Hilder and Boulter 1999; Barton and Dracup 2000; Kaeppeler 2000). These issues include: potential spread of the trait to closely related weedy species and impact on their ecology; possibility of nontarget effects on other diseases, pests, or beneficial microorganisms; potential health effects of the overexpression of antimicrobial proteins in foods; and possibility of promoting new pathogen strains with resistance to or that are able to overcome the novel engineered trait.

The spread of novel engineered genes from crop plants to weedy relatives has been demonstrated in some plant species through movement of pollen (Hails 2000; Wilkinson et al. 2000). Could a weedy sexually compatible relative of a crop species benefit from the potential introduction and expression of a disease-resistant phenotype? Weeds are known

to harbour inoculum of a wide range of fungal pathogens (Agrios 1997) and these pathogens may maintain a balance over weed growth. The potentially accelerated growth of a weedy species via introgression of the disease-resistant transgene, however, may be balanced by the reduction in primary inoculum that can be generated from weedy hosts adjacent to commercial crop fields, thereby reducing disease pressure. The extent to which the fitness of a weedy species may be enhanced by introgression of a disease-resistant phenotype has yet to be evaluated.

Nontarget effects on other diseases, pests, or beneficial microorganisms will have to be monitored in crop plants engineered to express antifungal or antimicrobial compounds. While unpredicted beneficial effects against other related fungal pathogens may be a positive aspect, an assessment of the effects on unrelated fungi, viruses, or bacteria may need to be conducted. It is unwieldy for researchers involved in the development of genetically engineered plants to screen against a multitude of diseases or pathogens common to that crop, an approach that may be taken by plant breeders during development of a new cultivar. The results in Table 1 demonstrate the specificity of the evaluation approach used for transgenic plants, which is conducted mostly under axenic or controlled environment conditions, and which infrequently includes more than one pathogen for challenge inoculation. A report of enhanced resistance to tobacco mosaic virus and potato virus X in a  $\beta$ -1,3-glucanase-deficient tobacco mutant (Iglesias and Meins 2000) suggests that plants overexpressing this enzyme to enhance fungal resistance should be tested for potentially enhanced susceptibility to viruses. Antisense transformation of tobacco to produce  $\beta$ -1,3-glucanase-deficient plants showed that these plants had increased deposition of callose in response to infection and had fewer lesions due to tobacco mosaic virus and tobacco necrosis virus (Beffa et al. 1996). How would the overexpression of antimicrobial compounds in the roots of genetically engineered plants alter their compatibility with mycorrhizae or beneficial endophytic fungi, or with various rhizosphere-colonizing microbes that could inhibit the development of soilborne pathogens? Evaluations of these potential effects have been conducted in a few studies (Vierheilig et al. 1993, 1995; Lottmann et al. 2000; Lukow et al. 2000; Lottmann and Berg 2001). No side effects have been found so far, except for one study in which cultivar-specific alterations in rhizosphere bacteria were found in a transgenic canola line (Siciliano and Germida 1999). Joint collaborations between molecular biologists and plant pathologists should foster the appropriate evaluations of these transgenic plants.

The potential of antimicrobial compounds to act as allergens or toxins when consumed by humans (Franck-Oberaspach and Keller 1997) would require that the guidelines established by the appropriate regulatory agencies be followed for the countries where the crops are grown and marketed (Kaeppeler 2000).

The possibility of selecting pathogen strains with resistance to the engineered trait may be increased with the widespread deployment of transgenic crops expressing specific antimicrobial compounds or that have broad-spectrum disease resistance. Fungal pathogens have demonstrated the capability for rapid change in genetic structure in the face



of selection forces, such as highly specific fungicides, major disease resistance genes, and environmental factors. The selection imposed by antimicrobial proteins, for example, could force the evolution of adaptive strategies in the pathogen to defend against the inhibitory compounds. Such a co-evolution has been proposed for chitinases (Bishop et al. 2000), in which adaptive functional modifications of the enzyme active site have occurred. Similarly, changes in sensitivity of pathogens to antimicrobial proteins overexpressed in transgenic plants could be selected. The use of combined genes that target different sites could reduce the selection pressure imposed on the pathogen. Genetically engineered plants with successfully enhanced disease resistance should not be viewed as a panacea and continual monitoring for unexpected events will be necessary.

### Future prospects

The tremendous scientific progress made since 1991 in genetic engineering of plants for enhanced resistance to fungal pathogens as described in this paper is an indication of the high level of interest in the scientific community on this subject. As the technology evolves toward the use of tissue-specific or pathogen-inducible promoters, the expression of engineered traits that are effective against a broad range of pathogens, and the utilization of synthetically derived peptides and of R genes, the impact on disease management will be enhanced. Evaluation of these transgenic plants for response to disease will need to be extended to field trials and appropriate agronomic data collected to ensure that this technology can be successfully implemented in farmer's fields to augment on-going disease management practices. Transgenic plants with enhanced disease resistance can become a valuable component of a disease management program in the future.

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### References

- Abad, M.S., Hakimi, S.M., Kaniewski, W.K., Rommens, C.M., Shulaev, V., Lam, E., and Shah, D.M. 1997. Characterization of acquired resistance in lesion-mimic transgenic potato expressing bacterio-opsin. *Mol. Plant-Microbe Interact.* 10: 635-645.
- Agrios, G.N. 1997. *Plant pathology*. 4th ed. Academic Press, New York.
- Alexander, D., Goodman, R.M., Gut-Rella, M., Glascock, C., Weymann, K., Friedrich, L., Maddox, D., Ahl-Goy, P., Luntz, T., Ward, E., and Ryals, J.A. 1993. Increased tolerance to two oomycete pathogens in transgenic tobacco expressing pathogenesis-related protein 1a. *Proc. Natl. Acad. Sci. U.S.A.* 90: 7327-7331.
- Asao, H., Nishizawa, Y., Arai, S., Sato, T., Hirai, M., Yoshida, K., Shinmyo, A., and Hibi, T. 1997. Enhanced resistance against a fungal pathogen *Sphaerotheca humuli* in transgenic strawberry expressing a rice chitinase gene. *Plant Biotechnol.* 14: 145-149.
- Baker, C.J., and Orlandi, E.W. 1995. Active oxygen in plant pathogenesis. *Annu. Rev. Phytopathol.* 33: 299-322.
- Baker, B., Zambryski, P., Staskawicz, B., and Dinesh-Kumar, S.P. 1997. Signalling in plant-microbe interactions. *Science (Washington, D.C.)*, 276: 726-733.
- Barker, S.J., and Tagu, D. 2000. The roles of auxins and cytokinins in mycorrhizal symbioses. *J. Plant Growth Regul.* 19: 144-154.
- Barton, J.E., and Dracup, M. 2000. Genetically modified crops and the environment. *Agron. J.* 92: 797-803.
- Beffa, R.S., Hofer, R.-M., Thomas, M., and Meins, F., Jr. 1996. Decreased susceptibility to viral disease of  $\beta$ -1,3-glucanase-deficient plants generated by antisense transformation. *Plant Cell*, 8: 1001-1011.
- Bent, A. 1996. Plant disease resistance genes: function meets structure. *Plant Cell*, 8: 1757-1771.
- Berna, A., and Bernier, F. 1997. Regulated expression of a wheat germin gene in tobacco: oxalate oxidase activity and apoplastic localization of the heterologous protein. *Plant Mol. Biol.* 33: 417-429.
- Beutini, P., Cosi, E., Pellegrini, M.G., Turbanti, L., Vendramin, G.G., and Buiatti, M. 1998. Modification of competence for in vitro response to *Fusarium oxysporum* in tomato cells. 3. PR-protein gene expression and ethylene evolution in tomato cell lines transgenic for phytohormone-related bacterial genes. *Theor. Appl. Genet.* 97: 575-583.
- Bi, Y.-M., Cammue, B.P.A., Goodwin, P.H., Krishna Raj, S., and Saxena, P.K. 1999. Resistance of *Botrytis cinerea* in scented geranium transformed with a gene encoding the antimicrobial protein Ace-AmP1. *Plant Cell Rep.* 18: 835-840.
- Bieri, S., Potrykus, I., and Fütterer, J. 2000. Expression of active barley seed ribosome-inactivating protein in transgenic wheat. *Theor. Appl. Genet.* 100: 755-763.
- Bishop, J.G., Dean, A.M., and Mitchell-Olds, T. 2000. Rapid evolution in plant chitinases: molecular targets of selection in plant-pathogen coevolution. *Proc. Natl. Acad. Sci. U.S.A.* 97: 5322-5327.
- Bliffeld, M., Mundy, J., Potrykus, I., and Fütterer, J. 1999. Genetic engineering of wheat for increased resistance to powdery mildew disease. *Theor. Appl. Genet.* 98: 1079-1086.
- Bohlmann, H. 1994. The role of thionins in plant protection. *Crit. Rev. Plant Sci.* 13: 1-16.
- Bolar, J.P., Norelli, J.L., Wong, K.-W., Hayes, C.K., Harman, G.E., and Aldwinckle, H.S. 2000. Expression of endochitinase from *Trichoderma harzianum* in transgenic apple increases resistance to apple scab and reduces vigor. *Phytopathology*, 90: 72-77.
- Boller, T. 1993. Antimicrobial functions of the plant hydrolases, chitinases and  $\beta$ -1,3-glucanases. In *Mechanisms of plant defense responses*. Edited by B. Fritig and M. Legrand. Kluwer Academic Press, Dordrecht. pp. 391-400.
- Brisson, L.F., Tenhaken, R., and Lamb, C.J. 1994. Function of oxidative cross-linking of cell wall structural proteins in plant disease resistance. *Plant Cell*, 6: 1703-1712.
- Broekaert, W.F., Terras, F.R.G., Cammue, B.P.A., and Osborn, R.W. 1995. Plant defensins: novel antimicrobial peptides as components of the host defense system. *Plant Physiol.* 108: 1353-1358.
- Broekaert, W.F., Cammue, B.P.A., De Bolle, M.F.C., Thevissen, K., De Samblanx, G.W., and Osborn, R.W. 1997. Antimicrobial peptides from plants. *Crit. Rev. Plant Sci.* 16: 297-323.
- Brogie, K., Chet, I., Holliday, M., Cressman, R., Biddle, P., Knowlton, S., Mauvais, C.J., and Brogie, R. 1991. Transgenic

- plants with enhanced resistance to the fungal pathogen *Rhizoctonia solani*. Science (Washington, D.C.), 254: 1194–1197.
- Brogliè, R., Brogliè, K., Roby, D., and Cher, I. 1993. Production of transgenic plants with enhanced resistance to microbial pathogens. In *Transgenic plants*. Vol. 1. Edited by S.-D. Kung, and R. Wu. Academic Press, New York. pp. 265–276.
- Bushnell, W.R., Somers, D.A., Giroux, R.W., Szabo, L.J., and Zeyen, R.J. 1998. Genetic engineering of disease resistance in cereals. Can. J. Plant Pathol. 20: 137–149.
- Cao, H., Li, X., and Dong, X. 1998. Generation of broad-spectrum disease resistance by overexpression of an essential regulatory gene in systemic acquired resistance. Proc. Natl. Acad. Sci. U.S.A. 95: 6531–6536.
- Cary, J.W., Rajasekaran, K., Jaynes, J.M., and Cleveland, T.E. 2000. Transgenic expression of a gene encoding a synthetic antimicrobial peptide results in inhibition of fungal growth in vitro and in planta. Plant Sci. 154: 171–181.
- Chamnongpol, S., Willekens, H., Moeder, W., Langebartels, C., Sandermann, H., Jr., Van Montagu, M., Inzé, D., and Van Camp, W. 1998. Defense activation and enhanced pathogen tolerance induced by H<sub>2</sub>O<sub>2</sub> in transgenic tobacco. Proc. Natl. Acad. Sci. U.S.A. 95: 5818–5823.
- Chang, M.-M., Chiang, C.C., Martin, M.W., and Hadwiger, L.A. 1993. Expression of a pea disease resistance response gene in the potato cultivar Shepody. Am. Potato J. 70: 635–647.
- Chen, W.P., Chen, P.D., Liu, D.J., Kynast, R., Friebe, B., Velazhahan, R., Muthukrishnan, S., and Gill, B.S. 1999. Development of wheat scab symptoms is delayed in transgenic wheat plants that constitutively express a rice thaumatin-like protein gene. Theor. Appl. Genet. 99: 755–760.
- Chong, D.K.X., and Langridge, W.H.R. 2000. Expression of full-length bioactive antimicrobial human lactoferrin in potato plants. Transgenic Res. 9: 71–78.
- Clausen, M., Kräuter, R., Schachermayr, G., Potrykus, I., and Sauter, C. 2000. Antifungal activity of a virally encoded gene in transgenic wheat. Nat. Biotechnol. 18: 446–449.
- Comménil, P., Belingheri, L., and Dehorte, B. 1998. Antilipase antibodies prevent infection of tomato leaves by *Botrytis cinerea*. Physiol. Mol. Plant Pathol. 52: 1–14.
- Constabel, P.C., Bertrand, C., and Brisson, N. 1993. Transgenic potato plants overexpressing the pathogenesis-related *STH-2* gene show unaltered susceptibility to *Phytophthora infestans* and potato virus X. Plant Mol. Biol. 22: 775–782.
- Cooley, M.B., Pathirana, S., Wu, H.-J., Kachroo, P., and Klessig, D.F. 2000. Members of the *Arabidopsis* *HRT/PPP8* family of resistance genes confer resistance to both viral and oomycete pathogens. Plant Cell, 12: 663–676.
- Cornelissen, B.J.C., and Melchers, L.S. 1993. Strategies for control of fungal diseases with transgenic plants. Plant Physiol. 101: 709–712.
- Crute, I.R., and Pink, D.A.C. 1996. Genetics and utilization of pathogen resistance in plants. Plant Cell, 8: 1747–1755.
- Dahleen, L.S., Okubara, P.A., and Blechl, A.E. 2001. Transgenic approaches to combat Fusarium head blight in wheat and barley. Crop Sci. 41. In Press.
- Dai, Z., Hooker, B.S., Anderson, D.B., and Thomas, S.R. 2000. Expression of *Acidothermus cellulolyticus* endoglucanase E1 in transgenic tobacco: biochemical characteristics and physiological effects. Transgenic Res. 9: 43–54.
- Datta, K., Velazhahan, R., Oliva, N., Ona, I., Mew, T., Khush, G.S., Muthukrishnan, S., and Datta, S.K. 1999. Overexpression of cloned rice thaumatin-like protein (PR-5) in transgenic rice plants enhances environmental-friendly resistance to *Rhizoctonia solani* causing sheath blight disease. Theor. Appl. Genet. 98: 1138–1145.
- Datta, K., Koukoliková-Nicola, Z., Baisakh, N., Oliva, N., and Datta, S.K. 2000. *Agrobacterium*-mediated engineering for sheath blight resistance of indica rice cultivars from different ecosystems. Theor. Appl. Genet. 100: 832–839.
- Datta, K., Tu, J., Oliva, N., Ona, I., Velazhahan, R., Mew, T.W., Muthukrishnan, S., and Datta, S.K. 2001. Enhanced resistance to sheath blight by constitutive expression of infection-related rice chitinase in transgenic elite indica rice cultivars. Plant Sci. 160: 405–414.
- De Bolle, M.F.C., Osborn, R.W., Goderis, I.J., Noe, L., Acland, D., Hart, C.A., Torrekens, S., Van Leuven, F., and Broekaert, W.F. 1996. Antimicrobial peptides from *Mirabilis jalapa* and *Amaranthus caudatus*: expression processing, localization and biological activity in transgenic tobacco. Plant Mol. Biol. 31: 993–1008.
- Deikman, J. 1997. Molecular mechanism of ethylene regulation of gene transcription. Physiol. Plant. 100: 561–566.
- De Jaeger, G., De Wilde, C., Eeckhout, D., Fiers, E., and Depicker, A. 2000. The plantibody approach: expression of antibody genes in plants to modulate plant metabolism or to obtain pathogen resistance. Plant Mol. Biol. 43: 419–428.
- Delaney, T.P., Uknes, S., Vernooij, B., Friedrich, L., Weymann, K., Negrotto, D., Gaffney, T., Gut-Rella, M., Kessman, H., Ward, E., and Ryals, J. 1994. A central role of salicylic acid in plant disease resistance. Science (Washington, D.C.), 266: 1247–1250.
- Delaney, T.P., Friedrich, L., and Ryals, J.A. 1995. *Arabidopsis* signal transduction mutant defective in chemically and biologically induced disease resistance. Proc. Natl. Acad. Sci. U.S.A. 92: 6602–6606.
- Dempsey, D.A., Silva, H., and Klessig, D.F. 1998. Engineering disease and pest resistance in plants. Trends Microbiol. 54: 54–61.
- Dempsey, D.A., Shah, J., and Klessig, D.F. 1999. Salicylic acid and disease resistance in plants. Crit. Rev. Plant Sci. 18: 547–575.
- Desiderio, A., Aracri, B., Leckie, F., Mattei, B., Salvi, G., Tigelaar, H., Van Roekel, J.S.C., Baulcombe, D.C., Melchers, L.S., De Lorenzo, G., and Cervone, F. 1997. Polygalacturonase-inhibiting proteins (PGIPs) with different specificities are expressed in *Phaseolus vulgaris*. Mol. Plant-Microbe Interact. 10: 852–860.
- De Wit, P.J.G.M. 1997. Pathogen avirulence and plant resistance: a key role for recognition. Trends Plant Sci. 2: 452–458.
- Dixon, R.A., Lamb, C.J., Masoud, S., Sewalt, V.J.H., and Paiva, N.L. 1996. Metabolic engineering: prospects for crop improvement through the genetic manipulation of phenylpropanoid biosynthesis and defense responses—a review. Gene (Amst.), 179: 61–71.
- Does, M.P., Houterman, P.M., Dekker, H.L., and Cornelissen, B.J.C. 1999. Processing, targeting, and antifungal activity of stinging nettle agglutinin in transgenic tobacco. Plant Physiol. 120: 421–431.
- Dong, X. 1998. SA, JA, ethylene and disease resistance in plants. Curr. Opin. Plant Biol. 1: 316–323.
- Donofrio, N.M., and Delaney, T.P. 2001. Abnormal callose response phenotype and hypersusceptibility to *Peronospora parasitica* in defense-compromised *Arabidopsis* *nim 1-1* and salicylate hydroxylase-expressing plants. Mol. Plant-Microbe Interact. 14: 439–450.
- Dumas, B., Freyssinet, G., and Pallert, K.E. 1995. Tissue-specific expression of germin-like oxalate oxidase during development and fungal infection of barley seedlings. Plant Physiol. 107: 1091–1096.

- Ellis, J., and Jones, D. 1998. Structure and function of proteins controlling strain-specific pathogen resistance in plants. *Curr. Opin. Plant Sci.* 1: 288-293.
- El Quakfaoui, S., Porvin, C., Brzezinski, R., and Asselin, A. 1995. A *Streptomyces* chitosanase is active in transgenic tobacco. *Plant Cell Rep.* 15: 222-226.
- Epple, P., Apel, K., and Bohlmann, H. 1997. Overexpression of an endogenous thionin enhances resistance of *Arabidopsis* against *Fusarium oxysporum*. *Plant Cell*, 9: 509-520.
- Evans, I.J., and Greenland, A.J. 1998. Transgenic approaches to disease protection: applications of antifungal proteins. *Pestic. Sci.* 54: 353-359.
- Fertig, S., and Hess, D. 1999. Expression of a chimeric stilbene synthase gene in transgenic wheat lines. *Transgenic Res.* 8: 179-189.
- Franck-Oberaspach, S.L., and Keller, B. 1997. Consequences of classical and biotechnological resistance breeding for food toxicology and allergenicity. *Plant Breed.* 116: 1-17.
- Gaffney, T., Friedrich, L., Vermooij, B., Negrotto, D., Nya, G., Uknes, S., Ward, E., Kessmann, H., and Ryals, J. 1993. Requirement of salicylic acid for the induction of systemic acquired resistance. *Science* (Washington, D.C.), 261: 754-756.
- Gao, A.-G., Halkimi, S.M., Minanck, C.A., Wu, Y., Woerner, B.M., Stark, D.M., Shah, D.M., Liang, J., and Rommens, C.M.T. 2000. Fungal pathogen protection in potato by expression of a plant defensin peptide. *Nat. Biotechnol.* 18: 1307-1310.
- Gelvin, S.B. 1998. The introduction and expression of transgenes in plants. *Curr. Opin. Biotechnol.* 9: 227-232.
- Govrin, E.M., and Levine, A. 2000. The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea*. *Curr. Biol.* 10: 751-757.
- Grayer, R.J., and Kokubun, T. 2001. Plant-fungal interactions: the search for phytoalexins and other antifungal compounds from higher plants. *Phytochemistry*, 56: 253-263.
- Grison, R., Grezes-Basset, B., Scheider, M., Lucante, N., Olsen, L., Leguay, J.-L., and Toppan, A. 1996. Field tolerance to fungal pathogens of *Brassica napus* constitutively expressing a chimeric chitinase gene. *Nat. Biotechnol.* 14: 643-646.
- Hails, R.S. 2000. Genetically modified plants—the debate continues. *Trends Ecol. Evol.* 15: 14-18.
- Hain, R., Reif, H.-J., Krause, E., Langebartels, R., Kindl, H., Vomam, B., Wiese, W., Schmeltzer, E., Schreier, P.H., Stöcker, R.H., and Stenzel, K. 1993. Disease resistance results from foreign phytoalexin expression in a novel plant. *Nature* (London), 361: 153-156.
- Hammerschmidt, R. 1999. Phytoalexins: what have we learned after 60 years? *Annu. Rev. Phytopathol.* 37: 285-306.
- Hammond-Kosack, K.E., and Jones, J.D.G. 1996. Resistance gene-dependent plant defense responses. *Plant Cell*, 8: 1773-1791.
- Hammond-Kosack, K.E., and Jones, J.D.G. 1997. Plant disease resistance genes. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48: 575-607.
- Hammond-Kosack, K.E., Harrison, K., and Jones, J.D.G. 1994. Developmentally regulated cell death on expression of the fungal avirulence gene *Avr 9* in tomato seedlings carrying the disease resistance gene *Cf-9*. *Proc. Natl. Acad. Sci. U.S.A.* 91: 10 444 - 10 449.
- Hammond-Kosack, K.E., Tang, S., Harrison, K., and Jones, J.D.G. 1998. The tomato *Cf-9* disease resistance gene functions in tobacco and potato to confer responsiveness to the fungal avirulence gene product *Avr 9*. *Plant Cell*, 10: 1251-1266.
- Hansen, G., and Wright, M.S. 1999. Recent advances in the transformation of plants. *Trends Plant Sci.* 4: 226-231.
- Hartley, M.R., Chaddock, J.A., and Bonness, M.S. 1996. The structure and function of ribosome inactivating proteins. *Trends Plant Sci.* 1: 254-260.
- He, X.-Z., and Dixon, R.A. 2000. Genetic manipulation of isoflavone 7-O-methyltransferase enhances biosynthesis of 4'-O-methylated isoflavonoid phytoalexins and disease resistance in alfalfa. *Plant Cell*, 12: 1689-1702.
- Hearsh, M.C. 2000. Hypersensitive response-related death. *Plant Mol. Biol.* 44: 321-334.
- Hedden, P., and Phillips, A.L. 2000. Manipulation of hormone biosynthetic genes in transgenic plants. *Curr. Opin. Biotechnol.* 11: 130-137.
- Hilder, V.A., and Boulter, D. 1999. Genetic engineering of crop plants for insect resistance—a critical review. *Crop Prot.* 18: 177-191.
- Hipskind, J.D., and Paiva, N.L. 2000. Constitutive accumulation of a resveratrol-glucoside in transgenic alfalfa increases resistance to *Phoma medicaginis*. *Mol. Plant-Microbe Interact.* 13: 551-562.
- Hoffman, T., Schmidt, J.S., Zheng, X., and Bent, A. 1999. Isolation of ethylene-insensitive soybean mutants that are altered in pathogen susceptibility and gene-for-gene disease resistance. *Plant Physiol.* 119: 935-949.
- Holtorf, S., Ludwig-Müller, J., Apel, K., and Bohlmann, H. 1998. High-level expression of a viscotoxin in *Arabidopsis thaliana* gives enhanced resistance against *Plasmodiophora brassicae*. *Plant Mol. Biol.* 36: 673-680.
- Monée, G. 1999. Engineered resistance against fungal pathogens. *Eur. J. Plant Pathol.* 105: 319-326.
- Monée, G., Melchers, L.S., Vleeshouwers, V.G.A.A., Van Roekel, J.S.C., and De Wit, P.J.G.M. 1995. Production of the AVR9 elicitor from the fungal pathogen *Cladosporium fulvum* in transgenic tobacco and tomato plants. *Plant Mol. Biol.* 29: 909-920.
- Howie, W., Joe, L., Newbigin, E., Suslow, T., and Dunsmuir, P. 1994. Transgenic tobacco plants which express the *chilA* gene from *Serratia marcescens* have enhanced tolerance to *Rhizoctonia solani*. *Transgenic Res.* 3: 90-98.
- Iglesias, V.A., and Meins, F. Jr. 2000. Movement of plant viruses is delayed in a  $\beta$ -1,3-glucanase-deficient mutant showing a reduced plasmodesmatal size exclusion limit and enhanced callose deposition. *Plant J.* 21: 157-166.
- Jach, G., Logemann, S., Wolf, G., Oppenheim, A., Chet, I., Schell, J., and Logemann, J. 1992. Expression of a bacterial chitinase leads to improved resistance of transgenic tobacco plants against fungal infection. *Bioprocess*, 1: 33-40.
- Jach, G., Görnhardt, B., Mundy, J., Logemann, J., Pinsdorf, E., Leah, R., Schell, J., and Mass, C. 1995. Enhanced quantitative resistance against fungal disease by combinatorial expression of different barley antifungal proteins in transgenic tobacco. *Plant J.* 8: 97-109.
- Johal, G.S., and Briggs, S.P. 1992. Reductase activity encoded by the *Hm1* disease resistance gene in maize. *Science* (Washington, D.C.), 258: 985-987.
- Jongedijk, E., Tigelaar, H., Van Roekel, J.S.C., Bres-Vloemans, S.A., Dekker, I., Van den Elzen, P.J.M., Cornelissen, B.J.C., and Melchers, L.S. 1995. Synergistic activity of chitinases and  $\beta$ -1,3-glucanases enhances fungal resistance in transgenic tomato plants. *Euphytica*, 85: 173-180.
- Kaeppler, H.F. 2000. Food safety assessment of genetically modified crops. *Agron. J.* 92: 793-797.
- Kamoun, S., van West, P., Vleeshouwers, V.G.A.A., de Groot, K.E., and Govers, F. 1998. Resistance of *Nicotiana benthamiana* to *Phytophthora infestans* is mediated by the recognition of the elicitor protein INF1. *Plant Cell*, 10: 1413-1425.

- Keller, H., Pamboukdjian, N., Ponchet, M., Poupet, A., Delon, R., Verrier, J.-L., Roby, D., and Ricci, P. 1999. Pathogen-induced elicitor production in transgenic tobacco generates a hypersensitive response and nonspecific disease resistance. *Plant Cell*, 11: 223-235.
- Kellmann, J.-W., Kleinow, T., Engelhardt, K., Philipp, C., Wegener, D., Schell, J., and Schreier, P.H. 1996. Characterization of two class II chitinase genes from peanut and expression studies in transgenic tobacco plants. *Plant Mol. Biol.* 30: 351-358.
- Kesarwani, M., Azam, M., Natarajan, K., Mehta, A., and Datta, A. 2000. Oxalate decarboxylase from *Collybia velutipes*. Molecular cloning and its overexpression to confer resistance to fungal infection in transgenic tobacco and tomato. *J. Biol. Chem.* 275: 7230-7238.
- Kikkert, J.R., Ali, G.S., Wallace, P.G., Reisch, B., and Reustle, G.M. 2000. Expression of a fungal chitinase in *Vitis vinifera* L. 'Merlot' and 'Chardonnay' plants produced by biolistic transformation. *Acta Hort.* (Wageningen), 528: 297-303.
- Kim, J.-K., Duan, X., Wu, R., Seok, S.J., Boston, R.S., Jang, I.-C., Eun, M.-Y., and Nahm, B.H. 1999. Molecular and genetic analysis of transgenic rice plants expressing the maize ribosome-inactivating protein b-32 gene and the herbicide resistance *bar* gene. *Mol. Breed.* 5: 85-94.
- Kitajima, S., and Sato, F. 1999. Plant pathogenesis-related proteins: molecular mechanisms of gene expression and protein function. *J. Biochem. (Tokyo)*, 125: 1-8.
- Knoester, M., Van Loon, L.C., Van den Heuvel, J., Hennig, J., Bol, J.F., and Linthorst, H.J.M. 1998. Ethylene-insensitive tobacco lacks nonhost resistance against soil-borne fungi. *Proc. Natl. Acad. Sci. U.S.A.* 95: 1933-1937.
- Koiwa, H., Kato, H., Nakatsu, T., Oda, J., Yamada, Y., and Sato, F. 1997. Purification and characterization of tobacco pathogenesis-related protein PR-5d, an antifungal thaumatin-like protein. *Plant Cell Physiol.* 38: 783-791.
- Kombrink, E., and Schmelzer, E. 2001. The hypersensitive response and its role in local and systemic disease resistance. *Eur. J. Plant Pathol.* 107: 69-78.
- Lagrimini, L.M., Vaughn, J., Erb, W.A., and Miller, S.A. 1993. Peroxidase overproduction in tomato: wound-induced polyphenol deposition and disease resistance. *HortScience*, 28: 218-221.
- Lagrimini, L.M., Joly, R.J., Dunlap, J.R., and Liu, T.-T.Y. 1997. The consequence of peroxidase overexpression in transgenic plants on root growth and development. *Plant Mol. Biol.* 33: 887-895.
- Lamb, C.J., Ryals, J.A., Ward, E.R., and Dixon, R.A. 1992. Emerging strategies for enhancing crop resistance to microbial pathogens. *Bio-Technology (N.Y.)*, 10: 1436-1445.
- Lassner, M., and Bedbrook, J. 2001. Directed molecular evolution in plant improvement. *Curr. Opin. Plant Biol.* 4: 152-156.
- Leckband, G., and Lörz, H. 1998. Transformation and expression of a stilbene synthase gene of *Vitis vinifera* L. in barley and wheat for increased fungal resistance. *Theor. Appl. Genet.* 96: 1004-1012.
- Lee, M.-W., Qi, M., and Yang, Y. 2001. A novel jasmonic acid-inducible rice *myb* gene associates with fungal infection and host cell death. *Mol. Plant-Microbe Interact.* 14: 527-535.
- Levine, A., Tenhaken, R., Dixon, R., and Lamb, C. 1994.  $H_2O_2$  from the oxidative burst orchestrates the plant hypersensitive disease resistance response. *Cell*, 79: 583-593.
- Li, Q., Lawrence, C.B., Xing, H.-Y., Babbitt, R.A., Bass, W.T., Maiti, I.B., and Everett, N.P. 2001. Enhanced disease resistance conferred by expression of an antimicrobial magainin analog in transgenic tobacco. *Planta* 212: 635-639.
- Liang, H., Maynard, C.A., Allen, R.D., and Powell, W.A. 2001. Increased *Septoria musiva* resistance in transgenic hybrid poplar leaves expressing a wheat oxalate oxidase gene. *Plant Mol. Biol.* 45: 619-629.
- Lin, W., Anuratha, C.S., Datta, K., Potrykus, I., Muthukrishnan, S., and Datta, S.K. 1995. Genetic engineering of rice for resistance to sheath blight. *Bio-Technology (N.Y.)*, 13: 686-691.
- Liu, D., Raghothama, K.G., Hasegawa, P.M., and Bressan, R. 1994. Osmotin overexpression in potato delays development of disease symptoms. *Proc. Natl. Acad. Sci. U.S.A.* 91: 1888-1892.
- Logemann, J., Jach, G., Tommerup, H., Mundy, J., and Schell, J. 1992. Expression of a barley ribosome-inactivating protein leads to increased fungal protection in transgenic tobacco plants. *Bio-Technology (N.Y.)*, 10: 305-308.
- Lorito, M., and Scala, F. 1999. Microbial genes expressed in transgenic plants to improve disease resistance. *J. Plant Pathol.* 81: 73-88.
- Lorito, M., Woo, S.L., D'Ambrosio, M., Harman, G.E., Hayes, C.K., Kubicek, C.P., and Scala, F. 1996. Synergistic interaction between cell wall degrading enzymes and membrane affecting compounds. *Mol. Plant-Microbe Interact.* 9: 206-213.
- Lorito, M., Woo, S.L., Fernandez, I.G., Colucci, G., Harman, G.E., Pintor-Toro, J.A., Filippone, E., Muccifora, S., Lawrence, C.B., Zoina, A., Tuzun, S., and Scala, F. 1998. Genes from mycoparasitic fungi as a source for improving plant resistance to fungal pathogens. *Proc. Natl. Acad. Sci. U.S.A.* 95: 7860-7865.
- Lottmann, J., and Berg, G. 2001. Phenotypic and genotypic characterization of antagonistic bacteria associated with roots of transgenic and non-transgenic potato plants. *Microbiol. Res.* 156: 75-82.
- Lottman, J., Heuer, H., de Vries, J., Mahn, A., Düring, K., Wackernagel, W., Smalla, K., and Berg, G. 2000. Establishment of introduced antagonistic bacteria in the rhizosphere of transgenic potatoes and their effect on the bacterial community. *FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol.* 33: 41-49.
- Lukow, T., Dunfield, P.F., and Liesack, W. 2000. Use of the T-RFLP technique to assess spatial and temporal changes in the bacterial community structure within an agricultural soil planted with transgenic and non-transgenic potato plants. *FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol.* 32: 241-247.
- Lund, S.T., Stall, R.E., and Klee, H.J. 1998. Ethylene regulates the susceptible response to pathogen infection in tomato. *Plant Cell*, 10: 371-382.
- Lusso, M., and Kuc, J. 1996. The effect of sense and antisense expression of the *PR-N* gene for  $\beta$ -1,3-glucanase on disease resistance of tobacco to fungi and viruses. *Physiol. Mol. Plant Pathol.* 49: 267-283.
- Maddaloni, M., Forlani, F., Balmas, V., Donini, G., Stasse, L., Corazza, L., and Motto, M. 1997. Tolerance to the fungal pathogen *Rhizoctonia solani* AG4 of transgenic tobacco expressing the maize ribosome-inactivating protein b-32. *Transgenic Res.* 6: 393-402.
- Maher, E.A., Bate, N.J., Ni, W., Elkind, Y., Dixon, R.A., and Lamb, C. 1994. Increased disease susceptibility of transgenic tobacco plants with suppressed levels of preformed phenylpropanoid products. *Proc. Natl. Acad. Sci. U.S.A.* 91: 7802-7806.
- Malehorn, D.E., Borgmeyer, J.R., Smith, C.E., and Shah, D.M. 1994. Characterization and expression of an antifungal zeamatin-like protein (*Zlp*) gene from *Zea mays*. *Plant Physiol.* 106: 1471-1481.
- Marchant, R., Davey, M.R., Lucas, J.A., Lamb, C.J., Dixon, R.A., and Power, J.B. 1998. Expression of a chitinase transgene in rose (*Rosa hybrida* L.) reduces development of blackspot disease (*Diplocarpon rosae* Wolf). *Mol. Breed.* 4: 187-194.
- Masoud, S.A., Zhu, Q., Lamb, C., and Dixon, R.A. 1996. Constitutive expression of an inducible  $\beta$ -1,3-glucanase in alfalfa reduces

- disease severity caused by the oomycete pathogen *Phytophthora megasperma* f. sp. *medicaginis*, but does not reduce severity of chitin-containing fungi. *Transgenic Res.* 5: 313–323.
- Mauch, F., and Staehelin, L.A. 1989. Functional implications of the subcellular localization of ethylene-induced chitinase and  $\beta$ -1,3-glucanase in bean leaves. *Plant Cell*. 1: 447–457.
- McDowell, J.M., and Dangl, J.L. 2000. Signal transduction in the plant immune response. *Trends Biochem. Sci.* 25: 79–82.
- Mehdy, M.C. 1994. Active oxygen species in plant defense against pathogens. *Plant Physiol.* 105: 467–472.
- Melchers, L.S., and Stuur, M.H. 2000. Novel genes for disease-resistance breeding. *Curr. Opin. Plant Biol.* 3: 147–152.
- Melchers, L.S., Sela-Buurlage, M.B., Vloemans, S.A., Woloshuk, C.P., Van Roekel, J.S.C., Pen, J., Van den Elzen, P.J.M., and Cornelissen, B.J.C. 1993. Extracellular targeting of the vacuolar tobacco proteins AP24, chitinase and  $\beta$ -1,3-glucanase in transgenic plants. *Plant Mol. Biol.* 21: 583–593.
- Métraux, J.-P. 2001. Systemic acquired resistance and salicylic acid: current state of knowledge. *Eur. J. Plant Pathol.* 107: 13–18.
- Mitsuhara, I., Matsufuru, H., Ohshima, M., Kaku, H., Nakajima, Y., Murai, N., Natori, S., and Ohashi, Y. 2000. Induced expression of sarcotoxin IA enhanced host resistance against both bacterial and fungal pathogens in transgenic tobacco. *Mol. Plant-Microbe Interact.* 13: 860–868.
- Mittler, R., Shulaev, V., and Lam, E. 1995. Coordinated activation of programmed cell death and defense mechanisms in transgenic tobacco plants expressing a bacterial proton pump. *Plant Cell*, 7: 29–42.
- Mohamed, R., Meilan, R., Ostry, M.E., Michler, C.H., and Strauss, S.H. 2001. Bacterio-opsin gene overexpression fails to elevate fungal disease resistance in transgenic poplar (*Populus*). *Can. J. For. Res.* 31: 268–275.
- Muhitch, M.J., McCormick, S.P., Alexander, N.J., and Hohn, T.M. 2000. Transgenic expression of the *TRI 101* or *PDR 5* gene increases resistance of tobacco to the phytotoxic effects of the trichothecene 4,15-diacetoxyscirpenol. *Plant Sci.* 157: 201–207.
- Murray, F., Llewellyn, D., McFadden, H., Last, D., Dennis, E.S., and Peacock, W.J. 1999. Expression of the *Talaromyces flavus* glucose oxidase gene in cotton and tobacco reduces fungal infection, but is also phytotoxic. *Mol. Breed.* 5: 219–232.
- Nakajima, H., Muranaka, T., Ishige, F., Akatsu, K., and Oeda, K. 1997. Fungal and bacterial disease resistance in transgenic plants expressing human lysozyme. *Plant Cell Rep.* 16: 674–679.
- Neuhaus, J.M. 1999. Plant chitinases (PR-3, PR-4, PR-8, PR-11). In *Pathogenesis-related proteins in plants*. Edited by S.K. Datta and S. Muthukrishnan. CRC Press, Boca Raton, Fla. pp. 77–105.
- Neuhaus, J.-M., Ahl-Goy, P., Hinz, U., Flores, S., and Meins, F., Jr. 1991. High-level expression of a tobacco chitinase gene in *Nicotiana glauca*. Susceptibility of transgenic plants to *Cercospora nicotianae* infection. *Plant Mol. Biol.* 16: 141–151.
- Neuhaus, K., Grsic-Rausch, S., Sauerteig, S., and Ludwig-Müller, J. 2000. *Arabidopsis* plants transformed with nitrilase 1 or 2 in antisense direction are delayed in clubroot development. *J. Plant Physiol.* 156: 756–761.
- Newell, C.A. 2000. Plant transformation technology. Developments and applications. *Mol. Biotechnol.* 16: 53–65.
- Nicholson, R.L., and Hammerschmidt, R. 1992. Phenolic compounds and their role in disease resistance. *Annu. Rev. Phytopathol.* 30: 369–389.
- Nielsen, K.K., Mikkelsen, J.D., Kragh, K.M., and Bojsen, K. 1993. An acidic class III chitinase in sugar beet: induction by *Cercospora beticola*, characterization, and expression in transgenic tobacco plants. *Mol. Plant-Microbe Interact.* 6: 495–506.
- Nishizawa, Y., Nishio, Z., Nakazono, K., Soma, M., Nakajima, E., Ugaki, M., and Hibi, T. 1999. Enhanced resistance to blast (*Magnaporthe grisea*) in transgenic Japonica rice by constitutive expression of rice chitinase. *Theor. Appl. Genet.* 99: 383–390.
- Nuutila, A.M., Ritala, A., Skadsen, R.W., Mannonen, L., and Kauppinen, V. 1999. Expression of fungal thermotolerant endo-1,4- $\beta$ -glucanase in transgenic barley seeds during germination. *Plant Mol. Biol.* 41: 777–783.
- O'Donnell, P.J., Calvert, C., Atzorn, R., Wasternack, C., Leyser, H.M.O., and Bowles, D.J. 1996. Ethylene as a signal mediating the wound response of tomato plants. *Science (Washington, D.C.)*, 274: 1914–1917.
- Oldach, K.H., Becker, D., and Lorz, H. 2001. Heterologous expression of genes mediating enhanced fungal resistance in transgenic wheat. *Mol. Plant-Microbe Interact.* 14: 832–838.
- Osusky, M., Zhou, G., Osuska, L., Hancock, R.E., Kay, W.W., and Misra, S. 2000. Transgenic plants expressing cationic peptide chimeras exhibit broad-spectrum resistance to phytopathogens. *Nat. Biotechnol.* 18: 1162–1166.
- Parashina, E.V., Serdobinskii, L.A., Kalle, E.G., Lavrova, N.V., Avetisov, V.A., Lunin, V.G., and Naroditskii, N.V. 2000. Genetic engineering of oilseed rape and tomato plants expressing a radish defensin gene. *Russ. J. Plant Physiol.* 47: 417–423.
- Park, C.-M., Berry, J.O., and Bruenn, J.A. 1996. High-level secretion of a virally encoded anti-fungal toxin in transgenic tobacco plants. *Plant Mol. Biol.* 30: 359–366.
- Powell, A.L.T., Stotz, H.U., Labavitch, J.M., and Bennett, A.B. 1994. Glycoprotein inhibitors of fungal polygalacturonases. In *Advances in molecular genetics of plant-microbe interactions*. Vol. 3. Edited by M.J. Daniels, J.A. Downie, and A.E. Osbourn. Kluwer Academic Publishers, Dordrecht. pp. 399–402.
- Powell, A.L.T., Van Kan, J., ten Have, A., Visser, J., Greve, L.C., Bennett, A.B., and Labavitch, J.M. 2000. Transgenic expression of pear PGIP in tomato limits fungal colonization. *Mol. Plant-Microbe Interact.* 13: 942–950.
- Punja, Z.K., and Raharjo, S.H.T. 1996. Response of transgenic cucumber and carrot plants expressing different chitinase enzymes to inoculation with fungal pathogens. *Plant Dis.* 80: 999–1005.
- Punja, Z.K., and Zhang, Y.-Y. 1995. Plant chitinases and their roles in resistance to fungal diseases. *J. Nematol.* 25: 526–540.
- Rajasekaran, K., Cary, J.W., Jacks, T.J., Stromberg, K.D., and Cleveland, T.E. 2000. Inhibition of fungal growth in plants and in vitro by transgenic tobacco expressing a bacterial nonheme chloroperoxidase gene. *Plant Cell Rep.* 19: 333–338.
- Rao, A.G. 1995. Antimicrobial peptides. *Mol. Plant-Microbe Interact.* 8: 6–13.
- Ray, H., Douches, D.S., and Hammerschmidt, R. 1998. Transformation of potato with cucumber peroxidase: expression and disease response. *Physiol. Mol. Plant Pathol.* 53: 93–103.
- Reymond, P., and Farmer, E.E. 1998. Jasmonate and salicylate as global signals for defense gene expression. *Curr. Opin. Plant Biol.* 1: 404–411.
- Roby, D., Broglie, K., Cressman, R., Biddle, P., Chet, I., and Broglie, R. 1990. Activation of a bean chitinase promoter in transgenic tobacco plants by phytopathogenic fungi. *Plant Cell*, 2: 999–1007.
- Rohini, V.K., and Rao, K.S. 2001. Transformation of peanut (*Arachis hypogaea* L.) with tobacco chitinase gene: variable response of transformants to leaf spot disease. *Plant Sci.* 160: 889–898.
- Rommens, C.M., and Kishore, G.M. 2000. Exploiting the full potential of disease-resistance genes for agricultural use. *Curr. Opin. Biotechnol.* 11: 120–125.

- Ryals, J.A., Neuenschwander, U.H., Willits, M.G., Molina, A., Steiner, H.-Y., and Hunt, M.D. 1996. Systemic acquired resistance. *Plant Cell*, 8: 1809–1819.
- Salmeron, J.M., and Vervoort, B. 1998. Transgenic approaches to microbial disease resistance in crop plants. *Curr. Opin. Plant Biol.* 1: 347–352.
- Schaffrath, U., Mauch, F., Freydl, E., Schweizer, P., and Dudler, R. 2000. Constitutive expression of the defense-related *Rir1b* gene in transgenic rice plants confers enhanced resistance to the rice blast fungus *Magnaporthe grisea*. *Plant Mol. Biol.* 43: 59–66.
- Schillberg, S., Zimmerman, S., Zhang, M.-Y., and Fischer, R. 2001. Antibody-based resistance to plant pathogens. *Transgenic Res.* 10: 1–12.
- Schweizer, P., Christoffel, A., and Dudler, R. 1999. Transient expression of members of the germin-like gene family in epidermal cells of wheat confers disease resistance. *Plant J.* 20: 541–552.
- Sela-Buurlage, M.B., Ponstein, A.S., Bres-Vloemans, S.A., Melchers, L.S., Van den Elzen, P.M.J., and Cornelissen, B.J.C. 1993. Only specific tobacco (*Nicotiana tabacum*) chitinases and  $\beta$ -1,3-glucanases exhibit antifungal activity. *Plant Physiol.* 101: 857–863.
- Shah, D.M. 1997. Genetic engineering for fungal and bacterial diseases. *Curr. Opin. Biotechnol.* 8: 208–214.
- Shah, D.M., Rommens, C.M.T., and Beachy, R.N. 1995. Resistance to diseases and insects in transgenic plants: progress and applications to agriculture. *Trends Biotechnol.* 13: 362–368.
- Shi, J., Thomas, C.J., King, L.A., Hawes, C.R., Posee, R.D., Edwards, M.L., Pallett, D., and Cooper, J.I. 2000. The expression of a baculovirus-derived chitinase gene increased resistance of tobacco cultivars to brown spot (*Alternaria alternata*). *Ann. Appl. Biol.* 136: 1–8.
- Shirasu, K., and Schulze-Lefert, P. 2000. Regulators of cell death in disease resistance. *Plant Mol. Biol.* 44: 371–385.
- Siciliano, S.D., and Germida, J.J. 1999. Taxonomic diversity of bacteria associated with the roots of field-grown transgenic *Brassica napus* cv. Quest compared to the non-transgenic *B. napus* cv. Excel and *B. rapa* cv. Parkland. *FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol.* 29: 263–272.
- Sitbon, F., Hennion, S., Little, C.H.A., and Sundberg, B. 1999. Enhanced ethylene production and peroxidase activity in IAA-overproducing transgenic tobacco plants is associated with increased lignin content and altered lignin composition. *Plant Sci.* 141: 165–173.
- Stark-Lorenzen, P., Nelke, B., Hännbier, G., Mühlbach, H.P., and Thormzik, J.E. 1997. Transfer of a grapevine stilbene synthase gene to rice (*Oryza sativa* L.). *Plant Cell Rep.* 16: 668–673.
- Staskawicz, B.J., Ausubel, F.M., Baker, B.J., Ellis, J.G., and Jones, J.D.G. 1995. Molecular genetics of plant disease resistance. *Science (Washington, D.C.)*, 268: 661–667.
- Staswick, P.E., Yuen, G.Y., and Lehman, C.C. 1998. Jasmonate signaling mutants of *Arabidopsis* are susceptible to the soil fungus *Pythium irregulare*. *Plant J.* 15: 747–754.
- Stichter, L., Mauch-Mani, B.N., and Métraux, J.P. 1997. Systemic acquired resistance. *Annu. Rev. Phytopathol.* 35: 235–270.
- Stirpe, F., Barbieri, L., Battelli, L.G., Soria, M., and Lippi, D.A. 1992. Ribosome-inactivating proteins from plants: present status and future prospects. *Bio-Technology (N.Y.)*, 10: 405–412.
- Strittmatter, G., and Wegner, D. 1993. Genetic engineering of disease and pest resistance in plants: present state of the art. *Z. Naturforsch.* 48: 673–688.
- Strittmatter, G., Janssens, J., Opsomer, C., and Botterman, J. 1995. Inhibition of fungal disease development in plants by engineering controlled cell death. *Bio-Technology (N.Y.)*, 13: 1085–1089.
- Swords, K.M.M., Liang, J., and Shah, D.M. 1997. Novel approaches to engineering disease resistance in crops. In *Genetic engineering*. Vol. 19. Edited by J.K. Setlow. Plenum Press, New York. pp. 1–13.
- Tabaeizadeh, Z., Agharbaoui, Z., and Harkak, H. 1999. Transgenic tomato plants expressing a *Lycopersicon chilense* chitinase gene demonstrate improved resistance to *Verticillium dahliae* race 2. *Plant Cell Rep.* 19: 197–202.
- Tabei, Y., Kitade, S., Nishizawa, Y., Kikuchi, N., Kayano, T., Hibi, T., and Akutsu, K. 1998. Transgenic cucumber plants harboring a rice chitinase gene exhibit enhanced resistance to gray mold (*Botrytis cinerea*). *Plant Cell Rep.* 17: 159–164.
- Takahashi, H., Chen, Z., Du, H., Liu, Y., and Klessig, D.F. 1997. Development of necrosis and activation of disease resistance in transgenic tobacco plants with severely reduced catalase levels. *Plant J.* 11: 993–1005.
- Takaichi, M., and Oeda, K. 2000. Transgenic carrots with enhanced resistance against two major pathogens, *Erysiphe heraclei* and *Alternaria dauci*. *Plant Sci.* 153: 135–144.
- Takatsu, Y., Nishizawa, Y., Hibi, T., and Akutsu, K. 1999. Transgenic chrysanthemum (*Dendranthema grandiflorum* (Ramat.) Kitamura) expressing a rice chitinase gene shows enhanced resistance to gray mold (*Botrytis cinerea*). *Sci. Hortic. (Amst.)*, 82: 113–123.
- Takken, F.L.W., and Joosten, M.H.A.J. 2000. Plant resistance genes: their structure, function and evolution. *Eur. J. Plant Pathol.* 106: 699–713.
- Tenhaken, R., Levine, A., Brisson, L.F., Dixon, R.A., and Lamb, C. 1995. Function of the oxidative burst in hypersensitive disease resistance. *Proc. Natl. Acad. Sci. U.S.A.* 92: 4158–4163.
- Tepfer, D., Bouteaux, C., Vigon, C., Aymes, S., Perez, V., O'Donohue, M.J., Huet, J.-C., and Pernollet, J.-C. 1998. Phytophthora resistance through production of a fungal protein elicitor ( $\beta$ -cryptogein) in tobacco. *Mol. Plant-Microbe Interact.* 11: 64–67.
- Terakawa, T., Takaya, N., Horiuchi, H., Koike, M., and Takagi, M. 1997. A fungal chitinase gene from *Rhizopus oligosporus* confers antifungal activity to transgenic tobacco. *Plant Cell Rep.* 16: 439–443.
- Terras, F.R.G., Eggermont, K., Kovaleva, V., Raikhel, N.V., Osborn, R.W., Kester, A., Rees, S.B., Torrekens, S., Van Leuven, F., Vanderleyden, J., Cammue, B.P.A., and Broekaert, W.F. 1995. Small cysteine-rich antifungal proteins from radish: their role in host defence. *Plant Cell*, 7: 573–588.
- Thomas, C.M., Jones, D.A., Parniske, M., Harrison, K., Balint-Kurtti, P.J., Hatzixanthis, K., and Jones, J.D.G. 1997. Characterization of the tomato *Cf-4* gene for resistance to *Cladosporium fulvum* identifies sequences that determine recognition specificity in *Cf-4* and *Cf-9*. *Plant Cell*, 9: 2209–2224.
- Thomma, B.P.H.J., Eggermont, K., Tierens, K.F.M.-J., and Broekaert, W.F. 1999a. Requirement of functional ethylene-insensitive 2 gene for efficient resistance of *Arabidopsis* to infection by *Botrytis cinerea*. *Plant Physiol.* 121: 1093–1101.
- Thomma, B.P.H.J., Nelissen, I., Eggermont, K., and Broekaert, W.F. 1999b. Deficiency in phytoalexin production causes enhanced susceptibility of *Arabidopsis thaliana* to the fungus *Alternaria brassicicola*. *Plant J.* 19: 163–171.
- Thompson, C., Dunwell, J.M., Johnstone, C.E., Lay, V., Ray, J., Schmitt, M., Watson, H., and Nisbet, G. 1995. Degradation of oxalic acid by transgenic oilseed rape plants expressing oxalate oxidase. *Euphytica*, 85: 169–172.



- Thomzik, J.E., Stenzel, K., Stöcker, R., Schreier, P.H., Hain, R., and Stahl, D.J. 1997. Synthesis of a grapevine phytoalexin in transgenic tomatoes (*Lycopersicon esculentum* Mill.) conditions resistance against *Phytophthora infestans*. *Physiol. Mol. Plant Pathol.* 51: 265-278.
- Van den Elzen, P.J.M., Jongedijk, E., Melchers, L.S., and Cornelissen, B.J.C. 1993. Virus and fungal resistance: from laboratory to field. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 342: 271-278.
- Van Loon, L.C., and Van Strien, E.A. 1999. The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. *Physiol. Mol. Plant Pathol.* 55: 85-97.
- Verberne, M.C., Verpoorte, R., Bol, J.F., Mercado-Blanco, J., and Linthorst, H.J.M. 2000. Overproduction of salicylic acid in plants by bacterial transgenes enhances pathogen resistance. *Nat. Biotechnol.* 18: 779-783.
- Vierheilig, H., Alt, M., Neuhaus, J.-M., Boller, T., and Wiemken, A. 1993. Colonization of transgenic *Nicotiana sylvestris* plants, expressing different forms of *Nicotiana tabacum* chitinase, by the root pathogen *Rhizoctonia solani* and by the mycorrhizal symbiont *Glomus mosseae*. *Mol. Plant-Microbe Interact.* 6: 261-264.
- Vierheilig, H., Alt, M., Lange, J., Gut-Rella, M., Wiemken, A., and Boller, T. 1995. Colonization of transgenic tobacco constitutively expressing pathogenesis-related proteins by the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae*. *Appl. Environ. Microbiol.* 61: 3031-3034.
- Vijayan, P., Shockey, J., Lévesque, C.A., Cook, R.J., and Browse, J. 1998. A role for jasmonate in pathogen defense of *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* 95: 7209-7214.
- Walton, J.D. 1994. Deconstructing the cell wall. *Plant Physiol.* 104: 1113-1118.
- Wang, P., Zoubenko, O., and Turner, N.E. 1998. Reduced toxicity and broad spectrum resistance to viral and fungal infection in transgenic plants expressing pokeweed antiviral protein II. *Plant Mol. Biol.* 38: 957-964.
- Wang, Y., Nowak, G., Culley, D., Hadwiger, L.A., and Fristensky, B. 1999. Constitutive expression of a pea defense gene *DRR206* confers resistance to blackleg (*Leptosphaeria maculans*) disease in transgenic canola (*Brassica napus*). *Mol. Plant-Microbe Interact.* 12: 410-418.
- Warrad, C., Kobiler, D., Dinoor, A., and Prusky, D. 1997. Pectate lyase of *Colletotrichum gloeosporioides* attacking avocado fruits—cDNA cloning and involvement in pathogenicity. *Physiol. Plant Pathol.* 50: 197-212.
- Weiler, E.W. 1997. Octadecanoid-mediated signal transduction in higher plants. *Naturwissenschaften*, 84: 340-349.
- Wilkinson, J.Q., Lanahan, M.B., Clark, D.G., Bleecker, A.B., Chang, C., Meyerowitz, E.M., and Klee, H.J. 1997. A dominant mutant receptor from *Arabidopsis* confers ethylene insensitivity in heterologous plants. *Nat. Biotechnol.* 15: 444-447.
- Wilkinson, M.J., Davenport, I.J., Charters, Y.M., Jones, A.E., Allainguillaume, J., Butler, H.T., Mason, D.C., and Raybould, A.F. 2000. A direct regional scale estimate of transgene movement from genetically modified oilseed rape to its wild progenitors. *Mol. Ecol.* 9: 983-991.
- Woloshuk, C.P., Meulenhoff, J.S., Sela-Buurlage, M., Van den Elzen, P.J.M., and Cornelissen, B.J.C. 1991. Pathogen-induced proteins with inhibitory activity toward *Phytophthora infestans*. *Plant Cell*, 3: 619-628.
- Wong, K.W., Harman, G.E., Norelli, J.L., Gustafson, H.L., and Aldwinckle, H.S. 1999. Chitinase-transgenic lines of 'Royal Gala' apple showing enhanced resistance to apple scab. *Acta Hortic. (Wagening.)*, 484: 595-599.
- Wu, G., Shortt, B.J., Lawrence, E.B., Levine, E.B., Fitzsimmons, K.C., and Shah, D.M. 1995. Disease resistance conferred by expression of a gene encoding H<sub>2</sub>O<sub>2</sub>-generating glucose oxidase in transgenic potato plants. *Plant Cell*, 7: 1357-1368.
- Wu, G., Shortt, B.J., Lawrence, E.B., Léon, J., Fitzsimmons, K.C., Levine, E.B., Raskin, L., and Shah, D.M. 1997. Activation of host defense mechanisms by elevated production of H<sub>2</sub>O<sub>2</sub> in transgenic plants. *Plant Physiol.* 115: 427-435.
- Yamamoto, T., Iketani, H., Ieki, H., Nishizawa, Y., Notsuka, K., Hibi, T., Hayashi, T., and Matsuta, N. 2000. Transgenic grapevine plants expressing a rice chitinase with enhanced resistance to fungal pathogens. *Plant Cell Rep.* 19: 639-646.
- Yang, Y., Shah, J., and Klessig, D.F. 1997. Signal perception and transduction in plant defense responses. *Genes Dev.* 11: 1621-1639.
- Yao, K., De Luca, V., and Brisson, N. 1995. Creation of a metabolic sink for tryptophan alters the phenylpropanoid pathway and the susceptibility of potato to *Phytophthora infestans*. *Plant Cell*, 7: 1787-1799.
- Yoshikawa, M., Tsuda, M., and Takeuchi, Y. 1993. Resistance to fungal diseases in transgenic tobacco plants expressing the phytoalexin elicitor-releasing factor,  $\beta$ -1,3-endoglucanase, from soybean. *Naturwissenschaften*, 80: 417-420.
- Yu, D., Liu, Y., Fan, B., Klessig, D.F., and Chen, Z. 1997. Is the high basal level of salicylic acid important for disease resistance in potato? *Plant Physiol.* 115: 343-349.
- Yu, D., Xie, Z., Chen, C., Fan, B., and Chen, Z. 1999. Expression of tobacco class II catalase gene activates the endogenous homologous gene and is associated with disease resistance in transgenic potato plants. *Plant Mol. Biol.* 39: 477-488.
- Yun, D.-J., Bressan, R.A., and Hasegawa, P.M. 1997. Plant antifungal proteins. In *Plant breeding reviews*, Vol. 14. Edited by J. Janick. John Wiley and Sons, New York. pp. 39-88.
- Zhang, Z., Collinge, D.B., and Thordal-Christensen, H. 1995. Germin-like oxalate oxidase, a H<sub>2</sub>O<sub>2</sub>-producing enzyme, accumulates in barley attacked by the powdery mildew fungus. *Plant J.* 8: 139-145.
- Zhu, Q., Maher, E.A., Masoud, S., Dixon, R.A., and Lamb, C.J. 1994. Enhanced protection against fungal attack by constitutive co-expression of chitinase and glucanase genes in transgenic tobacco. *Bio-technology (N.Y.)*, 12: 807-812.
- Zhu, B., Chen, T.H.H., and Li, P.H. 1995. Activation of two osmotin-like protein genes by abiotic stimuli and fungal pathogen in transgenic potato plants. *Plant Physiol.* 108: 929-937.
- Zhu, B., Chen, T.H.H., and Li, P.H. 1996. Analysis of late-blight disease resistance and freezing tolerance in transgenic potato plants expressing sense and antisense genes for an osmotin-like protein. *Planta*, 198: 70-77.
- Zook, M., Hohn, T., Bonnen, A., Tsuji, J., and Hammerschmidt, R. 1996. Characterization of novel sesquiterpenoid biosynthesis in tobacco expressing a fungal sesquiterpene synthase. *Plant Physiol.* 112: 311-318.
- Zoubenko, O., Uckun, F., Hur, Y., Chet, I., and Turner, N. 1997. Plant resistance to fungal infection induced by nontoxic pokeweed antiviral protein mutants. *Nat. Biotechnol.* 15: 992-996.

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